

EFFECTS OF PALM OIL-BASED SURFACTANT USED IN PESTICIDE FORMULATION ON TERRESTRIAL PLANTS

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ABSTRACT

Polyoxyethylene (20) sorbitan monooleate, or POE 20S, is one of palm-based surfactants commonly used as adjuvant in pesticide formulation. Toxicity study of POE 20S toward plants is still lacking since public is usually misinformed that adjuvant is non-toxic. Therefore, a study was conducted to investigate the effect of POE 20S on terrestrial plants, i.e., tomato and corn. At every one-week interval, aqueous solutions of POE 20S at 1%, 3%, 5%, 7% and 10% w/v were sprayed onto planted seeds. Germination time, number of seedlings survived, shoot length, shoot weight and visual symptoms of injury were determined and compared with control. The results showed that the first spray of all POE 20S concentrations did not inhibit the germination of both tomato and corn seeds. All seeds germinated and emerged. However, the next 10% spray treatment of POE 20S showed significantly lower survival in both plants due to necrosis and severe shrivelling of their leaves. In terms of germination time, shoot length and shoot weight, only tomato plants showed no significant differences between all concentrations tested and the control. We concluded that POE 20S is safe and not harmful to non-target terrestrial plants if appropriate dilution is used for pesticide formulation.

Keywords: ecotoxicity, palm oil-based surfactant, pesticide, polyoxyethylene (20) sorbitan monooleate, seed germination.

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INTRODUCTION

Tremendous benefits have been derived from the use of pesticides, especially in agriculture sector, where pesticides help to increase world food production by reducing losses from weeds, diseases and insect pests (Alavanja, 2009). In 2019 alone, approximately 2 million tonnes of pesticides were utilised worldwide, where China is the major user, followed by the United States of America (USA) and Argentina. By the year 2020, the global pesticide usage has been estimated to increase up to

3.5 million tonnes (Sharma *et al.*, 2019). Consumptions of pesticide also increase rapidly in developing countries in Southeast Asia, *e.g.*, Malaysia, Cambodia, Laos and Vietnam (Schreinemachers *et al.*, 2015), where Malaysia recorded 67 300 t pesticide consumption in 2017 (Müller, 2020). The use of pesticides was predicted to increase yearly due to agricultural activities (FAO, 2017).

The pesticide formulation is a mixture of active and other ingredients. Active ingredients function as the toxicant, which are capable of preventing, destroying, repelling or mitigating pests. Meanwhile, other ingredients include the solvent, carrier and adjuvant (Herzfeld and Sargent, 2011), which are inactive and with no toxic effect, but are added to pesticide formulations to improve the pesticide's ability to control pests (Abhilash and Singh, 2009). Surfactants are the major type of

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adjuvant (Krogh *et al.*, 2003), which helps to improve the emulsifying, dispersing, spreading, sticking or penetrating properties of pesticides (Miller and Westra, 1998) and enhances the effectiveness of pesticide application on foliage (Aumatell, 1996). On soils, surfactants act as agents responsible for increasing the pesticide mobility (Huggenberger *et al.*, 1973). Non-ionic surfactants are the most important type of surfactant that are employed in pesticide formulations (Seaman, 1990). This is because, non-ionic surfactants are compatible with most active ingredients and appropriate to use with systemic pesticides.

Recent oil palm downstream research and development (R&D) efforts have focused more on the development of products utilising various oleochemical derivatives, including palm-based surfactant (Parveez *et al.*, 2020). For example, one of the studies conducted by the Malaysian Palm Oil Board (MPOB) indicated that polyoxyethylene (20) sorbitan monooleate or POE 20S has the potential to be used as adjuvant in pesticide formulation (Ismail *et al.*, 1998; 2010; 2014) since it was observed that mixed surfactants of POE 20S and C₁₂-C₁₄ fatty alcohol ethoxylate exhibited superior emulsification efficiency and stability (Sumaiyah *et al.*, 2019). This surfactant is an oily liquid derived from ethoxylated sorbitan (a derivative of sorbitol) and esterified with fatty acids (Herzfeld and Sargent, 2011). At present, POE 20S is among the non-ionic surfactants that are commonly used in pesticide formulation (Ismail *et al.*, 1998; 2010; Sumaiyah *et al.*, 2019).

According to Norris (1982) and Ismail *et al.* (2014), most surfactants used in pesticide formulation, including POE 20S, are generally considered to be inert and non-phytotoxic. In some cases, surfactant may cause mild phytotoxicity but with no effect to overall growth, and in other cases, surfactant may inhibit plant growth when the concentrations increased (Bhat *et al.*, 1992). However, according to US EPA (1997), many consumers have a misleading impression of the term 'inert ingredient', and believe it is harmless. Numerous studies have found that despite the widespread assumption that adjuvants or inert ingredients are physically, chemically and biologically inactive, substantial evidence suggested that inert ingredients by themselves harm all non-target organisms studied (Mullin *et al.*, 2016).

As described by Fishel (2014), some plants are affected by surfactant because certain surfactants may be capable of causing phytotoxicity or injury in terrestrial plants. However, the phytotoxicity data on terrestrial plants are quite scattered and they are mainly measured for linear alkylbenzene sulphonate (LAS), a synthetic surfactant derived from mineral oil (Endo *et al.*, 1969; Luxmoore *et al.*, 1974; Ying, 2006) and no toxicity data of POE 20S towards terrestrial plants are reported anywhere.

Since POE 20S has been widely used as adjuvant in pesticides, it is essential to assess its toxicity in order to provide an estimate of its potential to cause harm on terrestrial plants. This is because, theoretically, active ingredient used should kill the target pest or weed, but there is also possibility that the surfactant used as adjuvant may cause toxicity to surrounding plants. Hence, the surfactant assessment toward terrestrial plants is relevant and necessary in order to obtain more toxicity data on available surfactant. This data can contribute in finding the tolerance limit of certain surfactants to terrestrial plants and thus, a comprehensive database of surfactant toxicity can be developed.

According to Asma Liyana *et al.* (2020), no toxicity study of surfactant toward Malaysian native terrestrial plants has been reported. Since MPOB has developed palm-based surfactants and formulated pesticides, it is the opportunity for MPOB to establish a facility for toxicity testing of these products toward terrestrial plants, especially to cater for the local research. The data generated will indirectly provide important information for reference by current and future generation. For that reason, the present study was performed to evaluate the toxicity effect of POE 20S, a palm oil-based surfactant on the germination and emergence of terrestrial plants' seeds, *i.e.*, corn and tomato under laboratory conditions.

MATERIALS AND METHODS

Plant Species

Seeds from two plant species, *Zea mays* or corn (represents monocotyledonous plant) and *Solanum lycopersicum* or tomato (represents dicotyledonous plant), purchased from a local supplier, were used in this study. These species were chosen because they are two of the test species recommended in the test guideline Organization for Economic Co-operation and Development (OECD) 208 - Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test (OECD 208, 2006). Besides, they are economically important, widely distributed, abundance and commonly used in research. Seeds used in this study were not treated with fungicides, insecticides or repellents prior to study initiation.

Soil Substrate

The soil used in the study was commercial sandy loam potting soil produced by local supplier. The soil was sent for elemental analysis (determination of nitrogen, carbon and sulphur content) before use in the experiment in order to ensure the required quality of soil used is consistent throughout the study. The properties of the soil were as follows: total nitrogen 1.34%, total carbon 44.00%, organic

carbon 41.17%, total sulphur 1.52% and pH 5.78. The most important criteria of the commercial potting soil used is that it must contain not less than 1.50% organic carbon.

Experimental Design and Test Conditions

The experimental design for this study followed a standard method by OECD, *i.e.*, test guideline OECD 208 - Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test (OECD 208, 2006), which consisted of a control and treatment groups. A control group, which received no test substance, was maintained to assure that effects observed are associated with exposure to the test substance and this group was identical in every aspect to the treatment groups except for exposure to the test substances. With the help of marked stick, seeds were sown at uniform depth of 2 cm and 4 cm for respective tomato and corn in a temperature-controlled laboratory. One and 2-3 seeds were planted per pot for tomato and corn, respectively. Each treatment of both species consisted of 40 replicated pots.

Tomato and corn required different environmental (light and temperature) conditions to grow. Therefore, a preliminary study was performed to obtain the suitable environmental conditions for both plants. For tomato seeds, growth tests were performed under laboratory conditions for 21 days with additional aquarium lamp mounted on the culture rack to obtain light intensity of 1500 ± 500 lux. The photoperiod was set for 16 hr light and 8 hr dark using timer, while growth temperature was set at $22^\circ\text{C} \pm 2^\circ\text{C}$. Corn seeds were planted in the same laboratory conditions as tomato. However, germinated corn seeds need warmer condition with average temperature of $25^\circ\text{C} \pm 5^\circ\text{C}$ and young corn plants with leaves need higher light intensity from natural sunlight (approximately 4000 ± 1000 lux) for 12 hr per day. During the growth period, tomato plants were watered using sub-irrigated planter system, where a cup filled with reverse osmosis water was placed under each test pot. Meanwhile, corn plants were watered by spraying manually two times a day with reverse osmosis water.

Application of Test Substance

Polyoxyethylene (20) sorbitan monooleate (POE 20S, Sigma-Aldrich) was used as a test substance in this study. POE 20S, also known as Tween 80, is generally used as a non-ionic surfactant and emulsifier in pesticide and cosmetic formulation. A purity of 98% and hydrophilic-lipophilic balance (HLB) of 10 is provided in the product specification. After seeds sowing, the solutions of different POE 20S concentrations were sprayed immediately onto the planted seeds in each pot using a manual hand

sprayer. POE 20S doses were applied again onto both plant species at a one-week interval. A sufficient dosage of surfactant solutions (approximately 3-5 mL per spray per pot) were sprayed into the pots to uniformly wet the planted area or emerged shoots and leaves. Concentrations tested were 0 (control), 1%, 3%, 5%, 7% and 10% w/v in aqueous solution. Selection of these concentrations was based on water solubility properties of the POE 20S, where 10% w/v was the highest concentration used since this is the maximum solubility level for POE 20S to be totally soluble in water (Chemical Book, 2016).

Observation and Measurement

The germination of tomato seeds was completed within 10 days of planting, while corn seeds germination was completed within shorter time, *i.e.*, seven days. The number of seedlings germinated were counted in each pot to determine the germination percentage (GP) (Mahmood and Usman, 2014) as in Equation (1):

$$\text{Germination percentage (GP)} = \frac{\text{Total number of seedlings germinated}}{\text{Total number of seeds planted}} \times 100 \quad (1)$$

Experiments were continued for another 11 days and seven days for tomato and corn, respectively, to observe differences in young plants' growth and toxicity symptoms. Besides, percentage of survivals of emerged plants was also calculated at the end of experiment, *i.e.*, on day 21 and 14 for tomato and corn, respectively as in Equation (2):

$$\text{Survival} = \frac{\text{Total number of live plants at the end of experiment}}{\text{Total number of emerged plants}} \times 100 \quad (2)$$

The endpoints seed germination, shoot length, and fresh and dry biomass were assessed for each species after 21 days and 14 days for tomato and corn plants, respectively. The test was considered valid only if more than 70% of seeds in the control had a successful germination. At harvest, shoots were cut at the shoot-root junction and the shoot length was measured. The fresh shoots were dried in an oven at $60^\circ\text{C} \pm 1^\circ\text{C}$ for 24 hr to determine the dry biomass.

Phytotoxicity Test

Germination and early growth of tomato and corn plants treated with POE 20S aqueous solution may show phytotoxicity in some treatments.

Therefore, all treatments were assessed for phytotoxicity potential using test guideline OECD 208 - Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test (OECD 208, 2006). Visual symptoms of treatments and control groups were observed and recorded. At harvest, visual phytotoxicity symptoms (e.g., chlorosis, necrosis, wilting, etc.) were determined based on leaf injury using a modified morphological score for phytotoxicity (Vanhala *et al.*, 2004) as in Table 1.

TABLE 1. MODIFIED MORPHOLOGICAL SCORE FOR PHYTOTOXICITY

Score	Effects/ symptoms
0	No symptom/ damage/ injury
10	Negligible symptom
20	Chlorosis or yellowing of the leaf tips
30	Chlorosis up to the whole lamina
40	Twisting leaves and stunting, but no burning
50	Wilting leaves and stunting, but no burning
60	Necrosis or browning/ burning of the leaf tips
70	Moderate necrosis up to the whole lamina
80	Severe necrosis of the whole leaves and stem
90	All leaves dry, shriveled and necrotic
100	Dead plant

Source: Vanhala *et al.* (2004).

Phytotoxicity symptoms were rated based on severity of leaf injury or plant damage by visual observation on plant morphology using phytotoxicity rating scale of 0 to 10 (Ambarish *et al.*, 2017) as in Table 2.

TABLE 2. PHYTOTOXICITY RATING SCALE

Rating	% Phytotoxicity (leaf injury/ damage)
0	No phytotoxicity
1	1-10
2	11-20
3	21-30
4	31-40
5	41-50
6	51-60
7	61-70
8	71-80
9	81-90
10	91-100

Source: Ambarish *et al.* (2017).

Based on phytotoxicity rating scale, percent phytotoxicity index (PPI) was computed using the Equation (3):

$$PPI = \frac{\text{Sum of all numerical ratings}}{\text{Total number of plants observed} \times \text{Maximum phytotoxicity rating}} \times 100 \quad (3)$$

Based on PPI, which measures the mean percent of leaf injury or plant damage, phytotoxicity levels of surfactants were determined as low, medium or high as in Table 3.

TABLE 3. DETERMINATION OF PHYTOTOXICITY LEVEL

PPI	Phytotoxicity level
0	Nil
1-5	Low
5-10	Medium
>10	High

Source: Ambarish *et al.* (2017).

Statistical Analysis

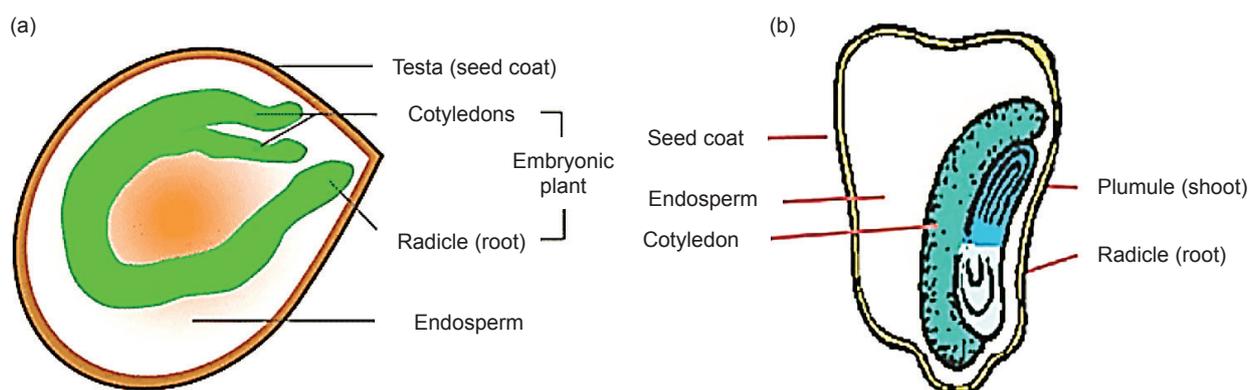
Data of seed germination, plants survival, germination time, shoot length and shoot weight were analysed using one-way analysis of variance (ANOVA) on SPSS 23 software (IBM Corporation, Armonk, New York, USA). The results are presented as mean ± standard deviation and data from the different treatment and control were compared by Tukey’s honestly significant difference (HSD) range test. Differences were considered significant when $p < 0.05$.

RESULTS AND DISCUSSION

Seed Germination

Seed germination is a resumption of embryo growth resulting in seed coat rupture and emergence of the young plant (Kozłowski and Pallardy, 1997). Figure 1 provides a simple diagram of tomato and corn seeds before germination. Percentage of seed germination is an important parameter for analysing the growth of seed under laboratory conditions. In this study, the seedling germination of tomato and corn fulfilled the validity criteria described by the standard guideline, where more than 70% of the seeds in the control group successfully germinated.

After the first spray treatment of 1%, 3%, 5%, 7% and 10% w/v POE 20S aqueous solution, *i.e.*, immediately after seeds have been planted, all tomato and corn seeds germinated and there were



Source: e-Limu (2015); Lichtenthaler (1996).

Figure 1. (a) Tomato, and (b) corn seeds before germination.

no significant adverse effects observed. Thus, the differences of GP between control and respective treatments were not statistically significant with GP value around 100%. The results indicated that the spraying of POE 20S solution immediately after seed planting did not inhibit the seed germination of both plants. In general, GP remained at a relatively high level for both plants at all five POE 20S concentrations tested. Even at the highest concentration tested, *i.e.*, 10%, there was no inhibition in the seed germination. This may be due to the fact that natural coating around the seeds act as a barrier between the embryo and its immediate environment. Therefore, the spraying of POE 20S solution immediately after seed planting may lead the solution to be absorbed by the seed coats and the seed coats simultaneously protected the embryo, thus, did not affect the germination (Di Salvatore *et al.*, 2008). Besides, Wong *et al.* (2001) also reported that seed germination is less sensitive to chemicals or toxicants than seed growth, in which the effect of exposure to the toxicant is higher only after the radical has emerged.

Survival and Growth Measurement

POE 20S solution was applied into the pot at one-week intervals throughout the experiment, regardless of whether or not germination occurred. Not all germinated seeds grew healthily until the end of the test period. In 5% w/v and 7% w/v treatments, only 85.0% and 62.5% of tomato, respectively, survived until end of the test period, while seeds treated with 10.0% w/v POE 20S exhibited the lowest survival of young tomato plants, *i.e.*, 30.0%. As for corn, all young plants in all treatments survived except plants treated with 10.0% w/v PEO 20S solution. At this concentration, only 42.5% of the corn plants survived, and the

rest were found dead at the end of the test. A trend was observed where an increase in concentration of tested surfactant decreased the percentage of seed germination and seedlings survival (Galvez *et al.*, 2018). In this study, as seen in both tomato and corn, the increasing concentrations of POE 20S solution also reduced the survival of the emerged plants. Significant differences ($p < 0.5$) in percentage of survived plants were found among the concentrations studied, *i.e.*, control, 1% and 3% > 5% and 7% > 10% in tomato; and control, 1%, 3%, 5% and 7% > 10% in corn. The survival percentages of emerged tomato and corn until end of the test period are shown in Figure 2.

As expected, the increase in surfactant concentrations resulted in a reduction of survival in emerged plants. Poor survival of emerged plants of both species in concentrated treatments (5%, 7% and 10% w/v) suggested that phytotoxicity symptoms may appear only at high POE 20S concentrations, *i.e.*, 5%, 7% and 10% w/v. When comparing the survival trend of both plants, it was clearly observed that corn survived better in POE 20S as compared to tomato. Ilic *et al.* (2015) explained that in the monocotyledonous seed, the embryo is enclosed by a thicker endosperm. It is possible that the thicker endosperm of corn seed protects the embryo from the effect of surfactant, particularly at the higher surfactant concentration.

Different indices, *i.e.*, germination time, shoot length and shoot weight of survived tomato and corn were measured and calculated after 21 and 14 days, respectively. The results are presented in Tables 4 and 5. As compared to control, the seedlings of tomato and corn showed delay of one to five days in their germination time with the increasing concentrations of POE 20S solution. According to Kimball *et al.* (2011), a delay in germination has direct influences on seedling

growth and physiology. As observed in this study, increasing POE 20S solution's concentration caused delay in seed germination and significantly affected both shoot length and shoot weight as

well. The observed data allow formulators to select appropriate concentration of POE 20S solution to be used as adjuvant in their products that will not pose bad effect on other non-target plants.

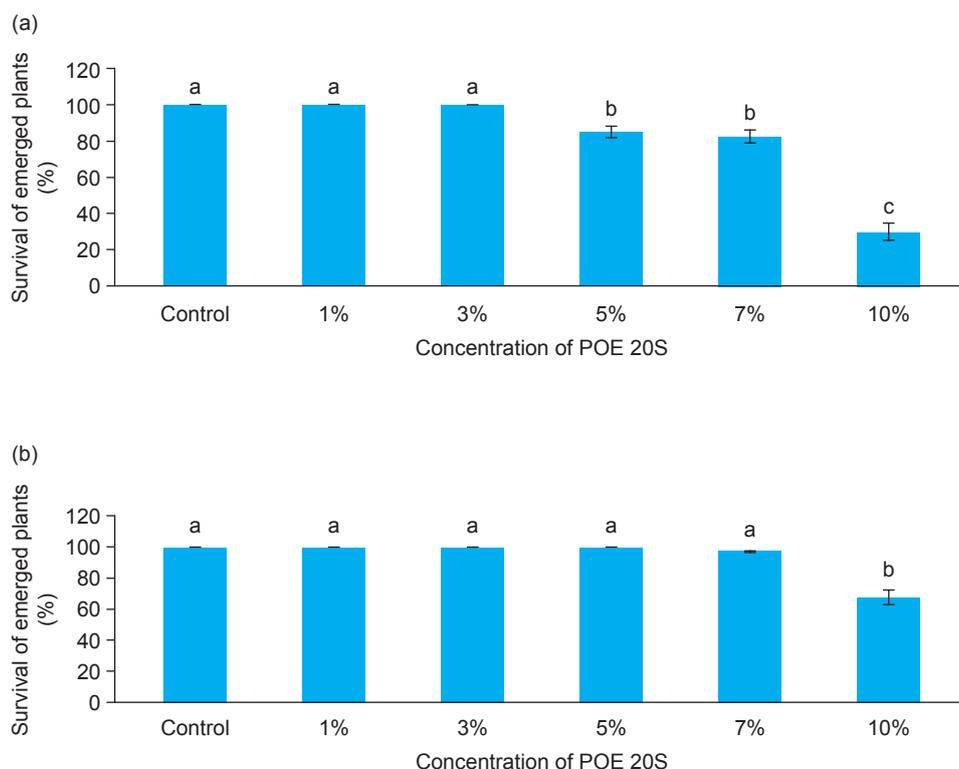


Figure 2. Percentage of survived emerged (a) tomato and (b) corn at the end of test, i.e., day 21 and 14, respectively. Error bars represent standard deviation. Columns with the same letter above them are not significantly different.

TABLE 4. MEAN ± STANDARD DEVIATION OF GERMINATION TIME, SHOOT LENGTH AND SHOOT DRY WEIGHT OF TOMATO PLANTS AT THE END OF TEST (Day 21)

POE 20S concentration (%)	Germination time (days)	Shoot length (cm)	Shoot dry weight (g)
0 (control)	5 ± 1	6.0 ± 0.3a	0.0048 ± 0.0009a
1	5 ± 1	5.4 ± 0.8b	0.0042 ± 0.0009b
3	6 ± 1	4.2 ± 0.7c	0.0030 ± 0.0005c
5	7 ± 1	3.8 ± 0.7cd	0.0027 ± 0.0005c
7	7 ± 1	3.4 ± 0.8d	0.0024 ± 0.0005cd
10	7 ± 1	3.3 ± 0.3d	0.0021 ± 0.0006d

Note: Means in the same column with the same letter are not significantly different.

TABLE 5. MEAN ± STANDARD DEVIATION OF GERMINATION TIME, SHOOT LENGTH AND SHOOT DRY WEIGHT OF CORN PLANTS AT THE END OF TEST (Day 14)

POE 20S concentration (%)	Germination time (days)	Shoot length (cm)	Shoot dry weight (g)
0 (control)	4 ± 1	17.5 ± 1.1a	0.0289 ± 0.0034a
1	4 ± 1	17.3 ± 1.0a	0.0277 ± 0.0028ab
3	4 ± 1	17.1 ± 0.7ab	0.0261 ± 0.0033b
5	5 ± 1	16.6 ± 1.0b	0.0229 ± 0.0035c
7	6 ± 1	14.6 ± 0.9c	0.0176 ± 0.0033d
10	7 ± 1	14.2 ± 1.0c	0.0166 ± 0.0012d

Note: Means in the same column with the same letter are not significantly different.

Phytotoxicity Test

Several differences in visible symptoms of toxicity were observed between control and treated groups at the end of experiments. In control, no detrimental symptoms were detected for both plants and so were the 1% and 3% w/v POE 20S for tomato. However, 5% and 7% w/v POE 20S resulted in moderate necrosis, where clear burning symptoms could be seen on tips of the leaves, while 10% w/v POE 20S contributed to severe drying of tomato plants towards the end of test (Figure 3).

Corn treated with 1% w/v POE 20S exhibited negligible symptoms, but most of the corn seedlings treated with 3% and 5% w/v POE 20S exhibited yellowish leaves, either on the tips or over the whole lamina. In 7% w/v POE 20S, mild necrosis could be seen on the tip of leaves and over the edge of leaves' lamina, while severe wrinkling and shrivelling of leaves could be found in the corn plants treated with 10% w/v POE 20S (Figure 4). Thus, this proves that symptoms of toxicity toward emerged seedlings are proportionate with the POE 20S concentrations tested.

Among the three parameters evaluated (seed germination, survival of germinated seeds and shoot growth), the clearest examples of toxicity effects were observed for shoot growth. These observations have been supported by Chang *et al.* (2015) who found that, since the shoot are the first target tissue to be exposed to the surfactants, the toxic symptoms appear to be greater for them compared to other parts of plant.

The toxicity symptoms observed were rated based on severity of leaves damage and were used to calculate PPI as in Table 6. From the results obtained, increased concentration of POE 20S solution resulted in an increased PPI level and toxicity effect in both monocotyledonous and dicotyledonous plants. As such, POE 20S solution can be declared as safe at lower concentrations, *i.e.*, 1% and 3% w/v POE 20S since there are no inhibitory effects on both species in the treatments and the toxicity level was also low. Similar results were found by Galvez *et al.* (2018), who revealed that POE 20S solution was among the least phytotoxic compound when tested with onion (monocotyledonous) and lettuce (dicotyledonous), where the germination index was between 80%-85% and with absence of toxicity symptom.

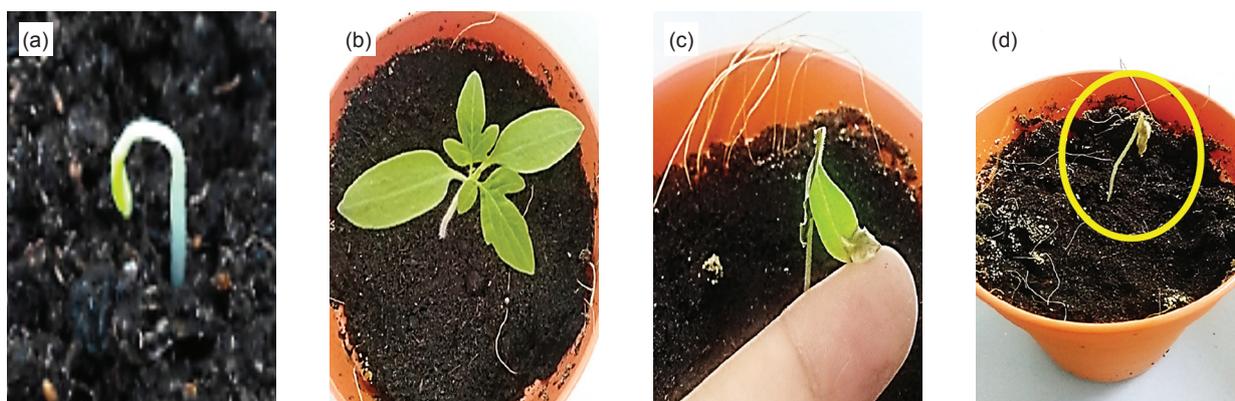


Figure 3. From left, (a) germinated tomato seed in control group on day 6, (b) tomato plant in control group on day 21, (c) moderate necrosis on tomato leaf treated with 7% POE 20S on day 21, and (d) severe drying of tomato plant treated with 10% POE 20S on day 21.

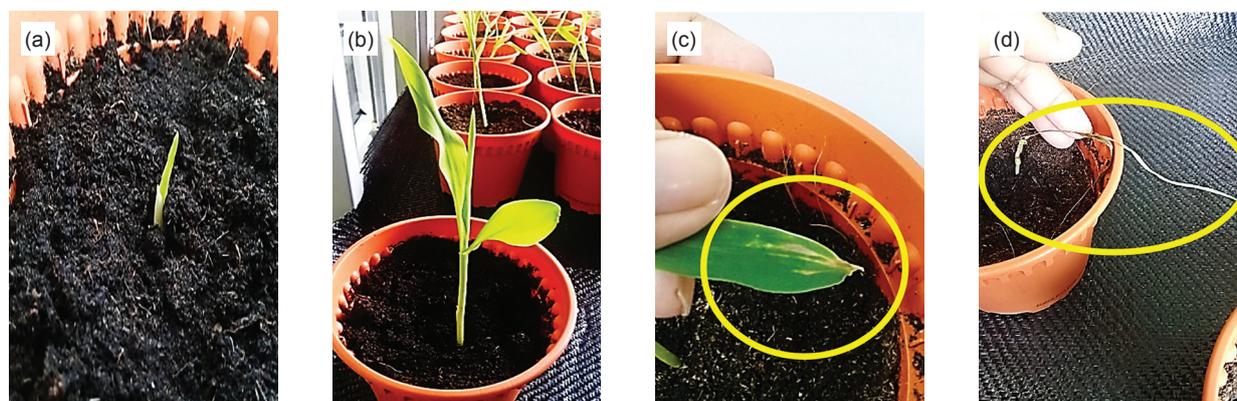


Figure 4. From left, (a) germinated corn seed in control group on day 5, (b) corn plant in control group on day 14, (c) mild necrosis on the tip of corn leaf treated with 7% POE 20S on day 14 and (d) severe shrivelling of corn plant treated with 10% POE 20S on day 14.

CONCLUSION

The results showed that higher concentration of POE 20S solution, *i.e.*, 5%, 7% and 10% w/v resulted in reduced survival of emerged plants of both species and increased level of phytotoxicity effect towards both dicotyledonous and monocotyledonous plants. With phytotoxicity level rated as low to medium for 1% to 10% w/v POE 20S solution, respectively, it is confirmed that, with appropriate dilution of POE 20S in pesticide formulation will not bring harm to non-target terrestrial plants. Data obtained from this study can be used as a reference to encourage manufacturers to select efficient vegetable-based surfactants for their pesticide formulation that will not bring harm to terrestrial environment, especially terrestrial plants.

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