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Allelophatic Effects of Orange Peel Extract on *Amaranthus hybridus* L. and *Pennisetum glaucum* (L.) R.Br.

Sani Sale[1]*, Aminu Idris Abubakar[1], Fatima Ja'afar[1], and Binta Abubakar[1]
[1] Department of Biological Sciences, Gombe State University, Nigeria.
*Corresponding Author's Email: sanisale20@yahoo.com; +2348029425225

Abstract
Weed control constitutes one of the biggest challenges in modern agriculture. Weed affects crops directly by competing for the limited resources thereby causing deficiency diseases and indirectly by housing pathogens and insect pests. The current control measures involve using synthetic herbicides which are believed to cause more harm than good to the environment. The way out is to resort to alternative bio-based herbicides, which are chief and eco-friendly. Consequently, this research investigated the potentials of orange peel crude extract in controlling both grasses and broad-leaved weeds. Orange peel was extracted using cold maceration methods in two separate solvents – water and methanol – and then divided into three concentrations. The allelophatic effects of these concentrations were tested against *Amaranthus hybridus* and *Pennisetum glaucum*. The results revealed that orange peel extract is effective in inhibiting germination and growth in the test plant. Fifty (50) % aqueous extract inhibited germination of *A. hybridus* and *P. glaucum* by 80 and 70% respectively, while 50% methanol extract inhibited germination of *A. hybridus* and *P. glaucum* 13% and 60% respectively. In addition, the treatments has reduced the seedling survivability with time. There was significant effects of the treatment on growth parameters of the test plants. Furthermore, the treatment has significantly affected the overall seedling vigour of the test plants. Fifty (50) % aqueous extract had the least seedling vigour in *A. hybridus*, while 30% of the same extract produced the least vigour in *P. glaucum*. The overall results indicated that aqueous extract of orange peel is more effective in inhibiting germination and growth of the test plants.

Keywords: bio-based herbicides, germination inhibition, seedling vigour, *A. hybridus* and *P. glaucum*

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Introduction

Weeds form one of the biggest challenges in agriculture because they compete with crops against the limited resources. They sometimes cause diseases and provide shelter for pathogens and insect pest causing worldwide annual loss of about US $95 billion (FAO, 2009) to farmers. Ability to control weed is very important as it ensures healthy crop free from insect pests and pathogen that would grow very fast thereby guaranteeing good agricultural outputs. The most common and effective way of controlling weed is by using synthetic herbicides. Although synthetic herbicides are more effective than other methods, they are often associated with certain environmental and health hazards such as soil and water pollution, and unsafe agricultural products (Mansour *et al*., 2014; Nicolopoulou-Stamati, 2016 and Florencia *et al*., 2017). In addition, synthetic herbicides are injurious to crops, they decrease crop quality (Silva *et al*., 2005), and may even affects non-target plants (Florencia *et al*., 2017).

Conserving natural environment and promoting human health, especially in pollution-prone agricultural areas, is a global concern. Recently, there is increasing efforts by researchers to reduce the amount of chemicals used in agricultural systems (Azizi and Fuji, 2006). Globally, there is a paradigm shift from synthetic agrochemicals to ecological and biological control methods (Azizi and Fuji, 2006). These new control methods are supposedly effective, cheap and ecofriendly.

Plants are the most targeted sources for bioactive compounds that would serve as safe and cheap alternative to synthetic chemicals. Such bioactive compounds include allelochemicals found in plants which work like herbicide by inhibiting the germination and/or growth of other plants (Fateh *et al*., 2012; Azizi and Fuji, 2006). Allelochemicals could be produced and stored in any plant parts (Tinnin and Muller, 2006) inform of phytotoxic products and are release from leaves, stem, roots, fruits and/or seeds (Fateh *et al*., 2012). They inhibit germination and/or growth of shoot/root of other plants (Hussain and Reigosa, 2011) by affecting some metabolic processes or nutrient cycles (Gholami *et al*., 2011), destroying the plant's usable source of nutrients or by causing some injurious effects on the crops (Fateh *et al*., 2012). One of the plant products with potential allelochemical is orange peel (Sharma and Tripathi, 2006; Ali and Celik, 2007). It has a good source of bioactive compounds (Larrauri *et al*., 1999) and is found in large quantity as orange byproduct (Manthey and Grohmann, 2001).

Phytochemical screening of orange peel indicates the presence of potential allelopathic chemicals (Khanh *et al*., 2007; Mandava, 1985) such as alkaloids, tannin, saphonins, terpenoids, cardiac glycosides (Arora and Kaur, 2013; Osarumwense *et al*., 2013; Shetty *et al*., 2016), anthroquinones, flavonoids (Gotmare and Gade, 2018), fixed oils and steroids (Kumar *et al*., 2014).

Various researches conducted indicated that orange peel extract inhibited the growth of radicle in lettuce (Ribeiro and Lima, 2012), and its essential oil was effective against *Euphorbia heterophylla* and *Ipomoea grandifolia* (Ribeiro and Lima, 2012). Also, the peel of other Citrus species like *Citrus aurantium* was found to be effective against *Amaranthus retroflexus* and *Cichorium intybus* (Mansour *et al*., 2014) and *Citrus junos* suppresses the germination of lettuce, and completely block the germination of tomato, celery, watercress. Furthermore, extract of Citrus junos suppresses the growth of weed like Chenopodium quinoa, Sonchus oleraceus and Digitaria ciliaris (Fujihara and Shimizu, 2003).

Considering the potentials of orange peel extract as herbicides and the availability of orange byproduct as waste, there is need to study its allelopathic effects extensively with the view to understanding its mechanisms of action and realizing its usability as bio-based herbicide. Consequently, this research intends to determine the allelopathic potentials of orange peel extract on germination and growth of Amaranthus hybridus and Pennisetum glaucum.

Materials and Methods

Study area

The research was conducted in Biological Sciences laboratory, Gombe State University, Gombe State, Nigeria.

Collection of Plant Materials

Fresh orange peel was collected from local vendors in Gombe markets in March, 2018; while the seeds of millet and amaranthus were bought from local farmers in Gombe, Gombe State Nigeria.

Extraction of plant material

The collected orange peels were rinsed with water, cut into small pieces, shade dried and grinded into powder using electric blender. Each ten 20g of the orange peel powder was the extracted with 200mL solvent (Shetty *et al*., 2016). Cold maceration method was used with two solvents – water and methanol – at room temperature and shaken continuously for 24h (Marinov-Serafimov,

2010). The solution was then filtered through Whatman filter paper. The filtrate was allowed to settle and the solvent was removed by evaporation using water bath. The extract was then dried, weighted and formulated to three concentrations of 50, 30 and 10% w/v, and 0% w/v was used as control.

Experimental setup

Ten (10) equal-sized seeds each of Amaranthus hybridus and Pennisetum glaucum were selected, washed and soaked in each concentration and then placed in a petri dishes containing moistened filter paper (Mansour et al., 2014). The whole setup was then incubated for seeds to germinate.

Data collection and analysis

Information on germination percentage and shoot and root growth were taken at the 3rd, 5th and 7th days after germination respectively. The data was analyzed using ANOVA and simple percentage. Inhibition (%) of germination for each treatment was calculated using the relation, %GI = [(C – T)/ C] * 100. Where %GI = % germination inhibition, C = control and T = treatment (Mansour et al., 2014) and seedling vigour index (SVI) was determined using the relation, SVI = RL + SL * GP, (Abdul-Baki and Anderson, 1973), where RL = mean root length, SL = mean shoot length and GP is germination percentage.

Results

Effects of *C. sinensis* peel extract on *P. glaucum*

The result indicated significant effect of *C. sinensis* peel extract on germination of *P. glaucum.* Fifty (50) % and 30% concentrations of aqueous extract of *C. sinensis* peel allowed only 30% and 40% germination respectively, whereas 100% germination was recorded in control (Fig. 1). The germination percentages decrease (from initial to final) across the treatments except control which increased from initial to final (Fig. 1).

There was significant effect ($p \leq 0.05$) of the extract on the growth of *P. glaucum* between treatments on seedling length (Table 1). Thirty (30) % aqueous extract has the lowest seedling growth (2.45cm) at the third day of germination, while 50% methanol extract has the highest (4.85cm). At the fifth day, 50% aqueous extract (2.30cm) had the least seedling length while 10% aqueous extract has the highest (4.86cm). At the seventh day also, 50% aqueous extract had least seedling growth (2.0cm) while 30% aqueous extract has the highest (5.28cm).

The result showed no significant differences ($p \geq 0.05$) between treatments on number of leaves at all stages of growth.

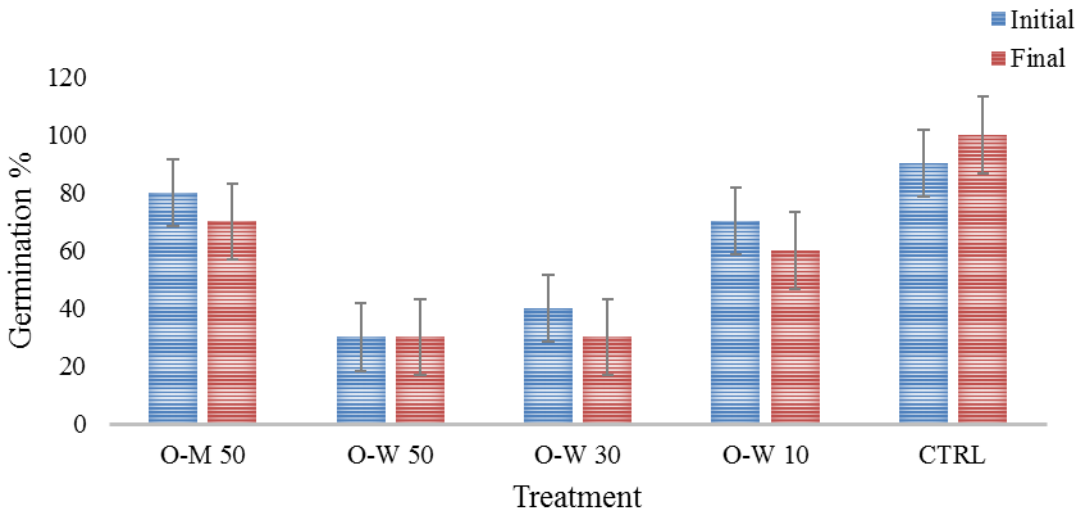

Figure 1: Germination percentage of *P. glaucum* seeds treated with *C. sinensis* peel extract
Key: O-M = Orange peel Methanol extract, O-W = Orange peel aqueous extract, CTRL=Control

There was significant difference ($p \leq 0.05$) between treatments on root length. Fifty (50) % methanol extract had the least root length (3.60cm) at the third day while control has the highest (6.70cm). At the fifth day, 30% aqueous extract had the least root length (4.55cm) followed by 10% while control had the highest (8.43cm). At the seventh day, 30% aqueous extract had the least length of root (5.78cm) while 50% methanol extract and control had the highest root length of 10.25 and 10.08cm respectively.

The results of germination inhibition further showed the effects of the extract on *P. glaucum* (Fig. 2a). Fifty (50) % concentration of aqueous extract was the most effective with 70% inhibition, followed by 30% of the same extract, while 50 % concentration of methanol extract was the least (30%). In addition, the extract has significantly affected the seedling vigour of *P. glaucum* as compared to the control (Fig. 2b). Fifty (50) % and 30% concentration of aqueous extract have the least vigour of 251 and 245 respectively, while control was the most vigorous (697.5).

Table 1: Effect of *C. sinensis* peel extract on *P. glaucum*.

Treatment	SL3 (cm)	SL5 (cm)	SL7 (cm)	NL3	NL5	NL7	RL3 (cm)	RL5 (cm)	RL7 (cm)
Methanol 50	4.85± 0.93^{a}	2.75± 1.48^{ab}	2.48± 0.81^{bc}	1.00± 0.00^{a}	1.00± 0.00^{a}	1.00± 0.00^{a}	3.60± 1.28^{a}	6.53± 1.19^{a}	10.25± 5.3^{a}
Aqueous 50	2.75± 1.10^{b}	2.30± 1.29^{b}	2.00± 1.02^{c}	1.00± 0.00^{a}	1.00± 0.00^{a}	1.00± 0.00^{a}	5.65± 2.64^{a}	6.78± 4.28^{a}	6.90± 5.58^{a}
Aqueous 30	2.45± 1.55^{b}	3.70± 2.18^{ab}	5.28± 0.99^{a}	1.00± 0.00^{a}	1.00± 0.00^{a}	1.00± 0.00^{a}	4.55± 2.35^{a}	4.55± 2.57^{a}	5.78± 2.18^{a}
Aqueous 10	3.60± 0.63^{ab}	4.88± 1.20^{a}	4.28± 2.56^{ab}	1.00± 0.00^{a}	1.00± 0.00^{a}	1.00± 0.00^{a}	6.10± 2.46^{a}	6.25± 2.85^{a}	9.40± 4.39^{a}
Control	2.98± 0.53^{b}	2.73± 0.22^{ab}	2.75± 0.24^{bc}	1.00± 0.00^{a}	1.00± 0.00^{a}	1.00± 0.00^{a}	6.70± 0.88^{a}	8.43± 0.94^{a}	10.08± 1.7^{a}

Means with the same alphabets are not significantly different from each other at $\alpha = 0.05$.
Key: SL= Seedling length, NL=Number of leaves, RL=Root length.

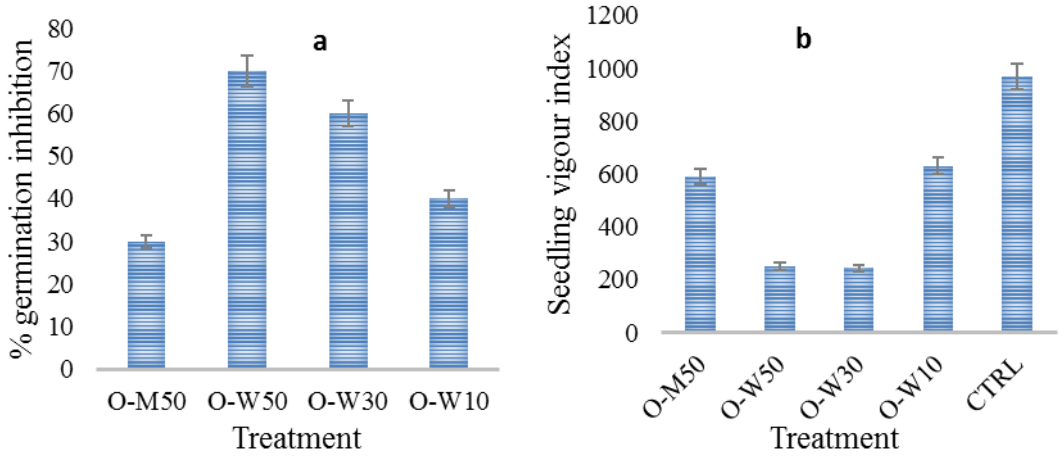

Figure 2: (a) percentage of germination inhibition**, and** (b) seedling vigour index of orange peel extract on *P. glaucum*
Key: O-M = Orange peel Methanol extract, O-W = Orange peel aqueous extract, CTRL=Control

Effect of *C. sinensis* peel extract on *A. hybridus*

The results indicated significant effect of *C. sinensis* peel extract on germination and growth of *A. hybridus*. Fifty (50) % concentration *C. sinensis* peel aqueous extract had the least germination of 20% whereas control had 90% germination (Fig. 3).

In addition, the results has shown some evidence of effect of the extract on the growth of *A. hybridus*. There was significant difference ($p \leq 0.05$) between treatments with respect to shoot and root length (Table 2). At the third day of germination, 30 % aqueous extract had the least seedling length (1.00cm), followed by 10% of the same extract, while 50% methanol extract and control have the highest length of 2.50 and 2.33 respectively. At the fifth day, also, 30% aqueous extract had the least seedling length (1.83cm) while 10% of the same extract and control have the highest seedling length of 2.93 and 2.90 respectively. At the seventh day, 50% and 30% aqueous extracts had the least seedling length of 1.57 and 1.65cm respectively, while 50% methanol extract had the highest length (2.93cm).

There was no significant difference in the number of leaves across the treatment and periods except at the fifth day where the control had the least number of leaves compared to other treatments.

The results showed that 50% of methanol extract and control have the least length of root (0.65cm) while 50% aqueous extract had the highest (1.83cm) at the third day. At the fifth day also, 50% methanol extract had the least length of root (0.25cm) while 30% aqueous extract had the highest (1.50cm). At the seventh day, no significant difference ($p \geq 0.05$) was observed between the treatments.

The results of germination inhibition further indicated the effects of the extract on *P. glaucum* (Fig. 4a). Fifty (50) % aqueous extract was the most effective with 87.5% inhibition, followed by 30 and 10% of the same extract, while 50% methanol extract had the least inhibition. Furthermore, the results has shown great differences between the treatments with respect to seedling vigour. Control is the most vigorous (238), followed by 50% methanol extract (220), 50% aqueous extract was the least vigorous (43).

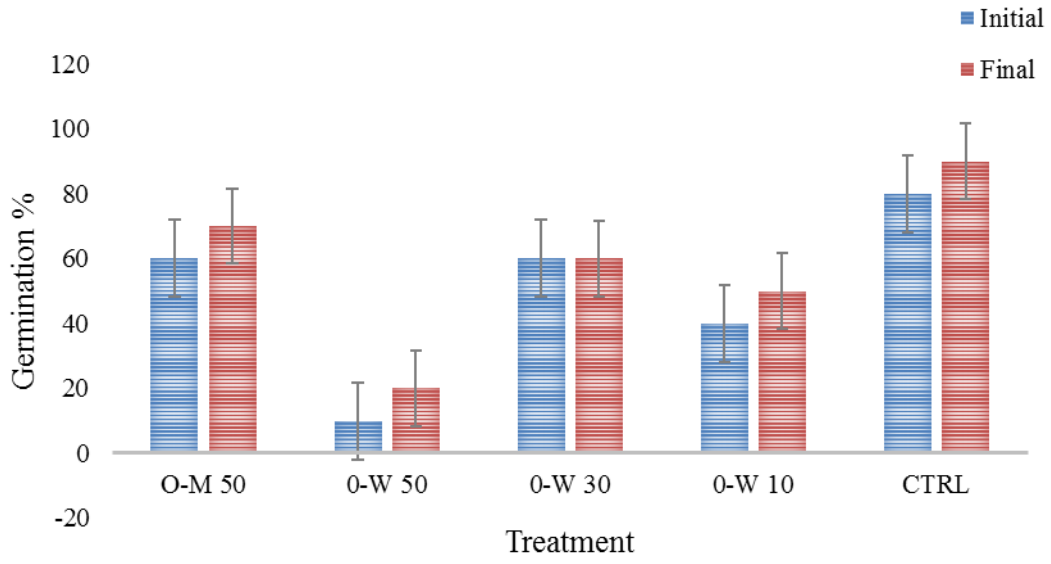

Figure 3: Germination percentage of *A. hybridus* seeds treated with *C. sinensis* peel extract
Key: O-M=Orange peel methanol extract, O-W= Orange peel aqueous extract, CTRL=Control

Table 2: Effect of *C. sinensis* peel extract on *A. hybridus*

Treatment	SL3 (cm)	SL5 (cm)	SL7 (cm)	NL3	NL5	NL7	RL3 (cm)	RL5 (cm)	RL7 (cm)
Methanol 50	2.50± 1.0^{a}	2.42± 0.3^{ab}	2.93± 0.4^{a}	1.75± 0.5^{a}	2.00± 0.0^{a}	1.75± 0.5^{a}	0.65± 0.17^{b}	0.25± 0.1^{b}	0.58± 0.3^{a}
Aqueous 50	2.40± 0.00^{a}	2.50± 0.0^{a}	1.57± 1.2^{b}	2.00± 0.0^{a}	2.00± 0.0^{a}	1.33± 0.6^{a}	1.83± 0.06^{a}	1.20± 0.0^{ab}	0.90± 0.3^{a}
Aqueous 30	1.00± 0.3^{b}	1.83± 0.6^{b}	1.65± 0.5^{b}	1.25± 0.5^{a}	1.75± 0.5^{a}	1.50± 1.0^{a}	1.40± 1.4^{ab}	1.50± 1.3^{a}	1.83± 2.1^{a}
Aqueous 10	1.50± 1.0^{ab}	2.93± 0.5^{a}	2.33± 0.7^{ab}	1.50± 1.0^{a}	2.00± 0.0^{a}	2.00± 0.0^{a}	1.10± 0.52^{ab}	1.23± 0.33^{ab}	1.28± 0.4^{a}
Control	2.33± 0.7^{a}	2.90± 0.5^{a}	2.08± 0.7^{ab}	2.00± 0.0^{a}	1.00± 0.0^{b}	2.00± 0.0^{a}	0.65± 0.3^{b}	1.25± 0.5^{ab}	0.53± 0.13^{a}

Means with the same alphabets are not significantly different from each other ($p \leq 0.05$)
Key: SL= Seedling length, NL=Number of leaves, RL=Root length.

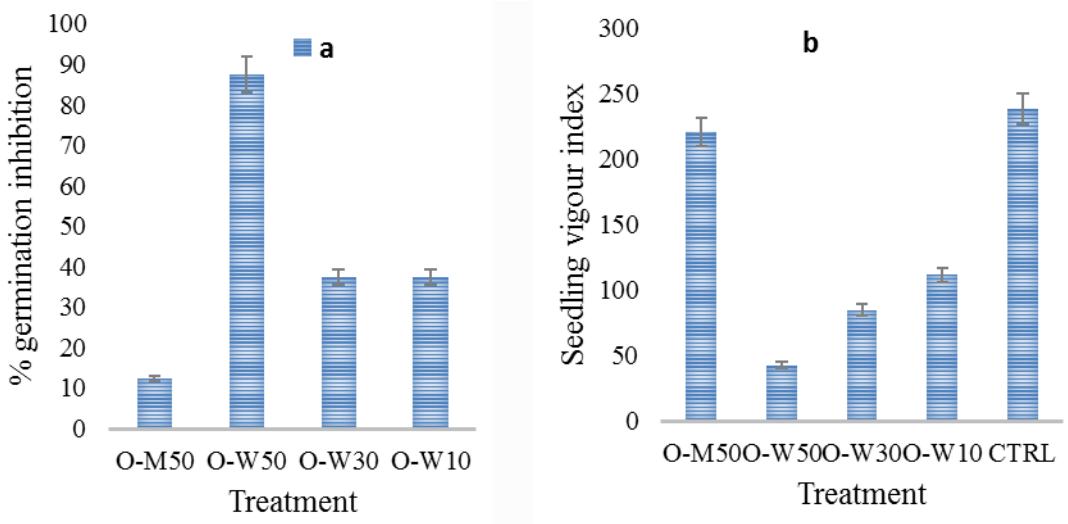

Figure 4: (a) Percentage of germination inhibition**,** and (b) Seedling vigour index of orange peel extract on *Amaranthus bybridus*
Key: O-M = Orange peel Methanol extract, O-W = Orange peel aqueous extract, CTRL=Control

Discussion

Effects of *C. sinensis* peel extract on *P. glaucum*

The treatment has inhibited germination of millet. Thirty (30) % germination was recorded for 50% and 30% aqueous extract and up to 100% for control. A similar research by Fateh *et al*. (2012) indicated that *Convolvulus arvensis* has germination inhibition effects on millet. Furthermore, germination percentage of all concentration decreased from initial germination to final seedling establishment, except control. This shows that the effect of the extract increases with time, and could even kill already-established seedlings of millet. This agrees with the work of Ribeiro and Lima, (2012) who found that essential oil from orange peel led to significant injuries that killed *Euphorbia heterophylla* seedlings.

The results also showed that the extract has affected the growth and development of

millet. Initially, 30% concentration of aqueous extract had the least shoot growth at the 3th day of germination.

However, at the 5th and 7th day, 50% concentration of aqueous extract had the least seedling growth. This shows that the effects of the extract last longer at higher concentrations and prolonged exposure to the extract enhances its allelophatic ability. The root length was also affected by the extract. Fateh *et al*. (2012) reported that *Convolvulus arvensis* has effects on the growth of millet. At the initial stage (3th day), 50% methanol extract was more effective, but at the 7th day, 30% aqueous extract was the most effective. This also shows the ability of the water to extract more allelophatic compound from orange peel than methanol. This agrees with the findings of Erukainre *et al.* (2016) who reported that type of solvent used in extraction determines the contents of the secondary metabolites.

The overall seedling vigour indicated that 50% and 30% concentrations of aqueous extract had the least vigour while control was the most vigorous. This indicated the efficacy of the extract to weaken the plant system.

Effect of *C. sinensis* peel extract on *A. hybridus*

The results indicated that the treatments has significant effects on the germination of *Amaranthus hybridus*. Fifty (50) % had the least germination percentage and hence the highest germination inhibition. This shows the ability of the extract to exact its effects on broad-leaved plants. This is in accordance with the work of Mansour *et al*. (2014) who reported that *Citrus aurantium* peel has reduced the germination of *Amaranthus retroflexus*. In addition, Extract of *Citrus junos* has been reported to strongly suppress the germination of lettuce (Fujihara and Shimizu, 2003).

Furthermore, the extract has additional effects on the growth and development of Amaranthus. The shoot length was severely affected by the extract. This is similar with the work of Mansour *et al*. (2014) who reported that *Citrus aurantium* peel has severe effects on growth of *Amaranthus retroflexus*. Similarly, Ribeiro and Lima (2012) reported that essential oil from orange peel has reduced the shoot size of *Euphorbia heterophylla*. Fifty (50) and 30% of aqueous extract had the least shoot length, but methanol extract had least effects on the plants. This indicates the ability of water to extract more active chemical constituents than methanol (Gotmare and Gade, 2018), which supports the work of Arora and Kaur (2013), who reported that saponins are absent in the orange peel extracted with methanol but present in aqueous extract. In addition, Erukainre *et al.* (2016) reported that solvent type determines the contents of an extract. However, the extracts of both solvents have no effects on the number of leaves. The methanol extract affected the growth of the roots at the initial stage. However, the effects subsided before 7th days of germination. This indicated that the root system resists the effects of the extract faster than shoot.

The results of the research showed the effects of the extract on the overall vigour of amaranth. 50% aqueous extract had the least vigour. This means the extract has the ability to weaken the plant as compared to the control. In a similar research, Otusanya *et al*. (2007) showed that *Tithonia diversifolia* has negative effects on germination, growth and fresh and dry mater of *Amaranthus cruentus*.

Conclusion

This research has revealed the ability of orange peel extract to inhibit the germination and suppress the growth of both narrow-leaved millet and broad-leaved Amaranthus. It further indicated the potentials of orange peel extract to be harnessed as board spectrum bio-based herbicide.

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