ASSESSING THE ALLELOPATHIC POTENTIAL OF MILK THISTLE *(Sonchus oleraceus* L.) On Germination and Seedling Growth of Red Rice *(Oryza punctata* Kotschy ex Steud.)

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ABSTRACT

Aqueous extracts of plants are used to control weeds being inexpensive and environment friendly. Present study was undertaken to investigate the effect of aqueous extracts of Sonchus oleraceus L. on the red rice (Oryza punctata Kotschy ex Steud.) emergence and initial seedling growth. Treatments included in the study were aqueous extract of leaves, stem and fruit of Sonchus oleraceus at varying concentrations 0.25, 0.50, 1, 2, 4 and 8 % (w/v) along with distilled water as a control. The allelochemicals present in the aqueous extracts showed stimulatory, inhibitory and hormetic responses depending upon the concentration and the plant part used for extraction. Results depicted maximum mean emergence time (5.26 days) and minimum germination index (1.67), germination percentage (40%), seedling fresh weight (59 g) and dry weight (8 g) of red rice with application of 8% aqueous extract of S. oleraceus fruit. However, 4% aqueous extract of S. oleraceus fruit produced minimum root length (5.71). On the basis of this experiment, it is concluded that 8% aqueous extract of S. oleraceus fruit can be used for controlling red rice weed.

Keywords: Aqueous extract, environment, allelochemicals, hermetic response, stimulatory

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INTRODUCTION

Rice (*Oryza sativa* L.) crop has a pivotal position in Pakistan's economy (Memon, 2013). Increasing population of Pakistan has resulted in more demand of rice production but weeds compete with rice crop for basic resources and reduce its yield (Ziska *et al.*, 2015). In Pakistan, weed intrusion in several ways badly lowers the production potential and yield of crops and responsible to cause about 17-50% losses (Abbas *et al.*, 2009). Weeds are harmful to rice crop, decrease grain production by 25-47% and straw yield by 13-38% through ways including interference with mineral uptake, their availability, changes in soil pH and hinder physiological processes in plants (Manandhar *et al.*, 2007).

Red rice (*Oryza punctata* Kotschy ex Steud.) is one of the major damaging, troublesome and dominant problem weed of rice crop worldwide. Chemical weed control is effective and time saving option but several weed species have developed resistance to herbicides and have become the root cause of ecological issues and selectivity of weeds growth (Kraehmer *et al.*, 2014). Increasing herbicide resistance in weeds has made chemical weed control an unsuitable method to sustain crop production (Abbas *et al.*, 2016a), allelopathy is quite effective to control resistant weeds (Abbas *et al.*, 2016b). It is very important to give attention to adopt some alternative natural ways that are less expensive, ecofriendly and not dependent on application of synthetic herbicides (Abbas *et al.*, 2016b). In this current scenario, allelopathically active plants should be tested for their herbicidal ability to suppress weed emergence and growth (Javaid and Khan, 2020; Javaid *et al.*, 2020). Different allelopathic compounds released from plant species have different modes of action on weed growth (Xuan *et al.*, 2005). Crops or weeds that release phenolic substances have the ability to suppress emergence and growth of weed specie (Fahmy *et al.*, 2012; Xu *et al.*, 2013). By applying the aqueous extracts of several weeds or crop plants that release allelochemicals can help us in controlling weeds in crops (Javaid *et al.*, 2020).

Several studies revealed that the weeds or crop plants having growth suppressing allelopathic potential showed greater tendency to act as alternatives of synthetic herbicides (Gomaa and Abd Elgawad, 2012; Fernandez-Aparicio *et al.*, 2008). Phenolics, terpenoids, carbohydrates, alkaloids, jasmonates, flavonoids, amino acids, momilactones, brassinosteroids, glucosinolates, hormones and hydroxamic acid are the distinctive compounds that hinder plant growth and development by altering the biochemical and physiological processes (Adaramola *et al.*, 2010; Asif, 2015). Plant growth, seedling emergence, germination, and biomass accumulation are slowed down by various allelochemicals by interference in physiological processes in plants (Zohaib *et al.*, 2016). *Sonchus oleraceus* L. (milk thistle) is a weed of winter season in Pakistan. The allelochemicals present in parts of *S. oleraceus* have the ability to inhibit emergence and initial seedling growth of radish (Aljubory *et al.*, 2010). The aqueous extract of *S. oleraceus* L. leaf exhibited a more inhibitory effect than those of other parts. Significant reduction was shown by 10% concentrated flower and fruit extracts (Aljubory *et al.*, 2010). Therefore, the present study was planned to evaluate the allelopathic potential of aqueous extracts of *S. oleraceus* at varying concentrations to manage *O. punctata*.

MATERIALS AND METHODS

An experiment was performed during summer 2017 to investigate the effect of water extracts of root, stem, fruit and flower of *S. oleraceus* (milk thistle) on *O. punctata* (red rice) in the Weed Science Laboratory, Department of Agronomy, University of Agriculture, Faisalabad, Pakistan. Completely randomized design (CRD) was followed with factorial arrangements having three replications of each treatment. Seeds of *O. punctata* weed were collected from the weed seed bank of the Department of Agronomy, University of Agriculture Faisalabad. The experiment comprised of aqueous extracts of leaves, stem and fruit of *S. oleraceus* at varying concentrations of 0.25, 0.50, 1, 2, 4 and 8 % (w/v) along with a control (0%).

Fully mature healthy plants of *S. oleraceus* were collected from Agronomic Farm of the University. The collected plants were washed and air dried under shade for two to three weeks until absolute drying was ensured. By using manual chopper, leaves, stem and fruits of the plants were chopped into small pieces and preserved at room temperature. Plant aqueous extracts were prepared in 1:10 w/v (weed part: distilled water) ratio. The dry chopped plant parts were dipped in distilled water at room temperature for overnight. Filter paper and manual sieve (3 mm) was used to separate the filtrate of these extracts and all the required concentrations were prepared by proper dilution. All the extracts were stored in bottles and then tagged by mentioning weed extract names and their concentrations on the bottles. These prepared weed aqueous extracts were preserved at room temperature in laboratory for further use in the experimental study. Ten seeds of red rice were placed in 9 cm diameter petri plates lined with double layer of Whatman filter paper. Initially, 7 ml of aqueous extract of each treatment was applied in petri plates. One treatment was maintained as control and only distilled water was applied during the whole study. The petri plates were sealed with paper tape to prevent them from dehydration. When the moisture content of filter paper was reduced, equal volumes of weed aqueous extracts and distilled water were added in petri plates as per treatment. Standard procedures were followed to record data regarding seed germination, time to 50% germination (Coolbear *et al.*, 1984; Farooq, 2004), mean germination time (Mordi Dezfuli *et al.*, 2008) and germination index (Association of Official Seed Analysis, 1990). The root and seedling biomass were recorded two weeks after germination. All the seedlings were oven dried at 60 °C for 48 hours to record seedling dry weight by following the formula proposed by Abdul-baki and Anderson (1973). The collected data were analyzed by using Statistics software (version, 8.1Statistix, Tallahassee, FL, USA) and least significant difference test (LSD) was used to compare the means of treatment at 5% probability level (Steel *et al.*, 1997).

RESULTS

Mean germination time (days)

Mean germination time of *O. punctata* was significantly influenced by the allelopathic influence of *S. oleraceus* weed organs (Table-1). The maximum value for mean emergence time (5.26 days) was noted with fruit aqueous extracts while the minimum value (2.21 days) was examined in stem extracts. The interaction effect of plant organs and concentration of aqueous extracts was significant, while, the results regarding varying concentrations were non-significant. The highest mean emergence time (5.26 days) was illustrated at 8% concentration by treating with fruit aqueous extracts while the lowest (2.21 days) was observed at 8% concentration by applying the stem aqueous extracts.

Germination index

The leaf, stem and fruit of *S. oleraceus* import imparted a statistically significant impact on red rice seeds germination index (Table-2). Maximum germination index (4.01) was produced by leaf extract while minimum (2.97) germination index was recorded in fruit extract. Among various concentrations of aqueous extract, the highest value of germination index (4.39) was noted at control (0%) while the lowest (2.12) at 8% concentration. Interaction effect of *S. oleraceus* parts and different concentrations produced significant influence on germination index of red rice. Maximum germination index (4.86) was observed at 0.25% under the influence of leaf aqueous extracts which was at par with distilled water control. While, minimum (1.69) germination index was recorded at 8% concentration with the application of fruit aqueous extracts.

Germination percentage

Red rice germination percentage was affected by the water extracts of *S. oleraceus* (Table-3). The highest (83.81%) germination percentage was noted with leaf aqueous extract which was statistically at par with stem aqueous extract, while the lowest mean value (68.571) was recorded with fruit aqueous extracts. Different concentrations of *S. oleraceus* significantly affected germination percentage of red rice. The maximum germination percentage (100 %) was recorded in the untreated check while, the minimum (46.67%) germination was recorded in 8% concentration. This showed that increase in concentration of weed aqueous extract was responsible for inhibition of seeds germination. The outcome of statistical analysis of interaction among the plant parts and concentration of extracts was also significant. The highest germination percentage (100%) was observed at control with leaf, stem and fruit aqueous extracts at par with 0.5% and 1% concentration with the application of leaf aqueous extracts. The minimum germination percentage (40.00 %) was noted at 8% concentrations with fruit aqueous extracts.

Time take to 50% germination (days)

The perusal of data given in table-4 indicated that *S. oleraceus* aqueous extracts imparted a significant allelopathic effect on time to 50% germination of red rice seeds. Seed of red rice not treated with water extract (0%) took least time (2.86 days) to complete 50% germination as compared with seed received 8% aqueous extract concentration (3.23 days). Parts of *S. oleraceus* produced non-significant effect on time to complete 50% germination of red rice. Interaction of plant parts and different concentrations produced significant impact on time taken by red rice to complete 50% germination. As compared to control, the highest significant mean value (2.91) was recorded at 8% concentration with the application of fruit aqueous extracts.

Seedling root length (cm)

Allelopathic effect posed by water extracts of *S. oleraceus* on the root length of red rice seedlings was found significant (Fig. 1). Seedling with highest length of root (5.93 cm) was examined under leaf aqueous extract of sow thistle and lowest root length (2.81 cm) seedlings was examined under fruit aqueous extract of weed. Among different concentrations of weeds extracts, the highest length of root (4.83 cm) was examined at concentration 1% while lowest length of root (3.94 cm) was recorded at concentration 0.25%. The interactive effect of plant part and concentration was more significant on root length of red rice seedlings as shown in Fig. 1. The maximum length of root (6.86 cm) was observed at 4% concentration by applying leaf aqueous extract of weed while the minimum length of root (2.04 cm) was examined at 4% concentration with fruit aqueous extract.

Seedling fresh weight (mg)

Leaf, stem and fruit aqueous extracts of *S*. *oleraceus* produced significant impact on red rice seedlings fresh weight (Fig. 2). Among the different plant parts aqueous extracts, leaf extracts produced maximum (110.94 mg) while minimum (75.21 mg) fresh weight was estimated with fruit extracts. Different concentration of water extracts of *S. oleraceus* produced significant influence on fresh weight of red rice. Seedlings with highest fresh weight (114.94 mg) were recorded at 0.25% concentration, while lowest fresh weight of seedlings (74.37 g) at 8% concentration of aqueous extracts of *S. oleraceus*. Plant parts × concentration also imparted significant effect on fresh weight of red rice. More fresh weight of seedling (158.67 mg) was recorded at 0.25% concentration with stem aqueous extracts while the lowest (59.00 g) was observed at 8% concentration by applying the fruit extracts.

Seedling dry weight (mg)

Red rice seedlings dry weight was significantly affected by the application of leaf, stem and fruit of *S. oleraceus* Fig. 3. The highest dry weight (12.51 mg) was obtained with stem water extract while the lowest (6.63 mg) was recorded when fruits extracts were applied. Among the different weed extract concentrations, the minimum dry weight (7.63 mg) was recorded at 4% concentration while the maximum dry weight was observed at 0.25%. The interactive effects of plant part and concentrations were not found significant.

DISCUSSION

The data showed that leaf and stem aqueous extracts were responsible for the reduction in time of emergence with increasing concentrations of extracts, while the fruit extracts promoted the seed germination at higher concentrations. This might be due to the presence of strong phytotoxic chemicals in the leaf and stem water extracts that reduced the mean germination time of seeds. These significant inhibitory results are analogous to Tanveer *et al.*, (2008, 2010) who reported noticeable inhibitory effects of water extracts of different parts of *Alternanthera* species. The leaves of *Alternanthera* species contain more inhibitory chemicals than any other plant part. Higher concentrations of weed aqueous extracts showed inhibitory effects towards germination index and significantly inhibit the germination of red rice. Our findings are also in line with the results of Kadioglue *et al.* (2005) and Tanveer *et al.* (2008, 2010) who revealed that the decrease in germination index of rice seeds by the application of aqueous extracts of two weeds namely *Alternanthera sessilis* and A*lternanthera philoxeroides.* Maximum inhibition of germination index and increase in emergence time of rice seeds was recorded in the aforesaid experiments. The higher concentrations of all the plant parts showed inhibitory effects towards germination percentage. Dongre and Yadav (2005) and Tanveer *et al.* (2010) also reported similar inhibitory special effects posed by water extracts of different parts of *Alternanthera* species. The leaves of *Alternanthera* species contain more inhibitory chemicals than any other plant part. Our results are in line to the findings of Dongre and Yadav (2005) and Tanveer *et al.* (2008 and 2010) who reported evident inhibitory effects posed by the water extracts of different parts of *Alternanthera* species. The leaves of *Alternanthera* species contain more inhibitory chemicals than any other plant part. This phytotoxic behavior of weeds was responsible for maximum delay in germination of seeds.

Results showed that overall, more inhibitory effects wereshown by fruit extracts comparatively while leaf and stem extracts on root length. This might be possible due to the presence of more phytotoxic substances in fruit extracts as compared to leaf and stem. The results regarding the allelopathic behavior of aqueous extract different parts of sow thistle on the root length were analogous obtained by Aljubory *et al.* (2010). These results are however contradictory to those of Park *et al.*, 2011 who reported the negative effects of higher concentrations of *S. oleraceus* stem extracts on root length. This contradiction in results can be attributed to the differences in the test crop, conditions from where samples were collected for preparation of extracts. Nadeem *et al.*(2020b) studied the effect of aqueous extracts of various parts of *C. tinctorius* on the root length of barnyard grass. Results revealed that minimum root length of barnyard grass was produced by produced by safflower leaves aqueous extract whereas, seedlings with lengthiest roots were noted by application of stem aqueous extracts of *C. tinctorius*.

Our results showed that fruit aqueous extracts act as inhibitory agents that causes reduction in fresh weight. The reduction in seedling growth might be the cause of reduction in fresh weight of seedlings. These findings are also supported by the experiments of Hassan *et al.* (2014) who confirmed that *S. oleraceus* exhibited strong allelopathic behavior towards the crops and weeds by decreasing the germination and seedling growth of test plants through the action of allelochemicals and phenols. According to Nadeem *et al.*(2020a) who reported that all the concentrations of *C. tinctorius* enhance the time to complete 50% emergence of *O. punctata* with 8% concentration.

Present findings revealed that *S. oleraceus* fruit extracts contain more phytotoxic substances that reduced red rice seedling shoot and root length and subsequently causes reduction in seedling dry weight. In agreement with these results are those of Hu and Zhang (2013) who reported that leaf extracts of *Chromolaena odorata* posed a more pronounced inhibitory effects on seed germination and seedling growth of different herbaceous plants as compared to root extracts.

CONCLUSION

It is concluded from this research that leaf, stem and fruit water extracts of *S. oleraceus* possess intrinsic phytotoxic potential against red rice weed germination and seedling growth. Higher concentration of all plant part extracts was more inhibitory than their low concentration. The overall inhibition in the germination and seedling growth of red rice was highest by fruit water extract at 8% concentration. The phytoregulatory substances in *S. oleraceus* aqueous extract could be exploited as a potential bio-herbicide for the eco-friendly management of red rice; one of the most troublesome weeds of the world.

Table-1. Allelopathic effects of aqueous extracts of *S. oleraceus* weed on mean emergence time (MET) of red rice seeds.

Plant parts	Mean emergence time							Mean
	Concentration							
	Control	**0.25%**	**0.5%**	**1%**	**2%**	**4%**	**8%**	
Leaf	3.98def	3.91d-g	3.95def	3.84e-h	3.75e-i	3.68e-i	3.56f-j	**4.12b**
Stem	4.06cde	3.92d-g	3.45hij	3.24jk	3.61f-j	2.87k	2.21l	**3.81c**
Fruit	3.36ij	3.50g-j	3.45hij	4.50b	4.29bcd	4.47bc	5.26a	**4.12a**
Mean	**3.80^{NS}**	**3.77**	**3.61**	**3.86**	**3.88**	**3.67**	**3.68**	

LSD (5%): Concentration =0.25, Plant parts = 1.65, Concentration × plant parts = 0.438

Mean sharing the same letter did not differ from each other at 5% probability level

Table-2. Allelopathic effects of aqueous extracts of *S. oleraceus* weed on germination index of red rice seeds.

Plant parts	Germination index							Mean
	Concentration							
	Control	**0.25%**	**0.5%**	**1%**	**2%**	**4%**	**8%**	
Leaf	4.80a	3.86a-e	4.86a	4.27a-d	3.97a-e	3.52a-f	2.77e-h	**4.01a**
Stem	4.47abc	3.39b-f	3.59a-f	3.11c-h	3.00d-i	2.36fgh	1.91gh	**3.12b**
Fruit	3.88a-e	3.33c-g	2.94d-h	2.97d-h	2.42fgh	3.58a-f	1.69h	**2.97b**
Mean	**4.39a**	**3.52b**	**3.80ab**	**3.45b**	**3.13b**	**3.15b**	**2.12c**	

LSD (5%): Concentration = 0.823, Plant part = 0.539, Concentration × plant parts = 1.42

Mean sharing the same letter did not differ from each other at 5% probability level

Table-3. Allelopathic effects of aqueous extracts of *S. oleraceus* weed on germination percentage of red rice seeds.

Plant parts	Germination percentage							Mean
	Concentration							
	Control	**0.25%**	**0.5%**	**1%**	**2%**	**4%**	**8%**	
Leaf	100.00a	86.67abc	100.00a	100.00a	66.67c-f	80.00a-d	53.33efg	**83.81a**
Stem	100.00a	93.33ab	90.00abc	80.00a-d	73.33b-e	60.00d-g	46.47fg	**77.61a**
Fruit	100.00a	86.67abc	66.67c-f	73.33b-e	53.33efg	60.00d-g	40.00g	**68.57b**
Mean	**4.39a**	**3.52b**	**3.80ab**	**3.45b**	**3.13b**	**3.15b**	**2.12c**	

LSD (5%): Concentration = 12.50, Plant part = 4.84, Concentration × plant parts = 20.39

Mean sharing the same letter did not differfrom each other at 5% probability level

Table-4. Allelopathic effects of aqueous extracts of *S. oleraceus* weed on time to 50% germination of red rice seeds.

Plant parts	Time to 50% germination (T_{50})							Mean
	Concentration							
	Control	**0.25%**	**0.5%**	**1%**	**2%**	**4%**	**8%**	
Leaf	3.33a	1.41g	2.27def	2.66a-f	2.50b-f	2.87a-d	2.91a-d	**2.67^{NS}**
Stem	3.25ab	2.91a-d	1.91fg	2.42c-f	2.65a-f	2.67a-f	2.87a-d	**2.48**
Fruit	3.11abc	1.41g	2.50a-f	2.41c-f	2.25def	2.76a-e	2.91a-d	**2.35**
Mean	**2.86ab**	**2.61bc**	**1.58d**	**2.39c**	**2.47bc**	**2.87ab**	**3.23a**	

LSD (5%): Concentration = 0.45, Plant parts = 0.29, Concentration × plant parts = 0.78

Mean sharing the same letter did not differ from each other at 5% probability level

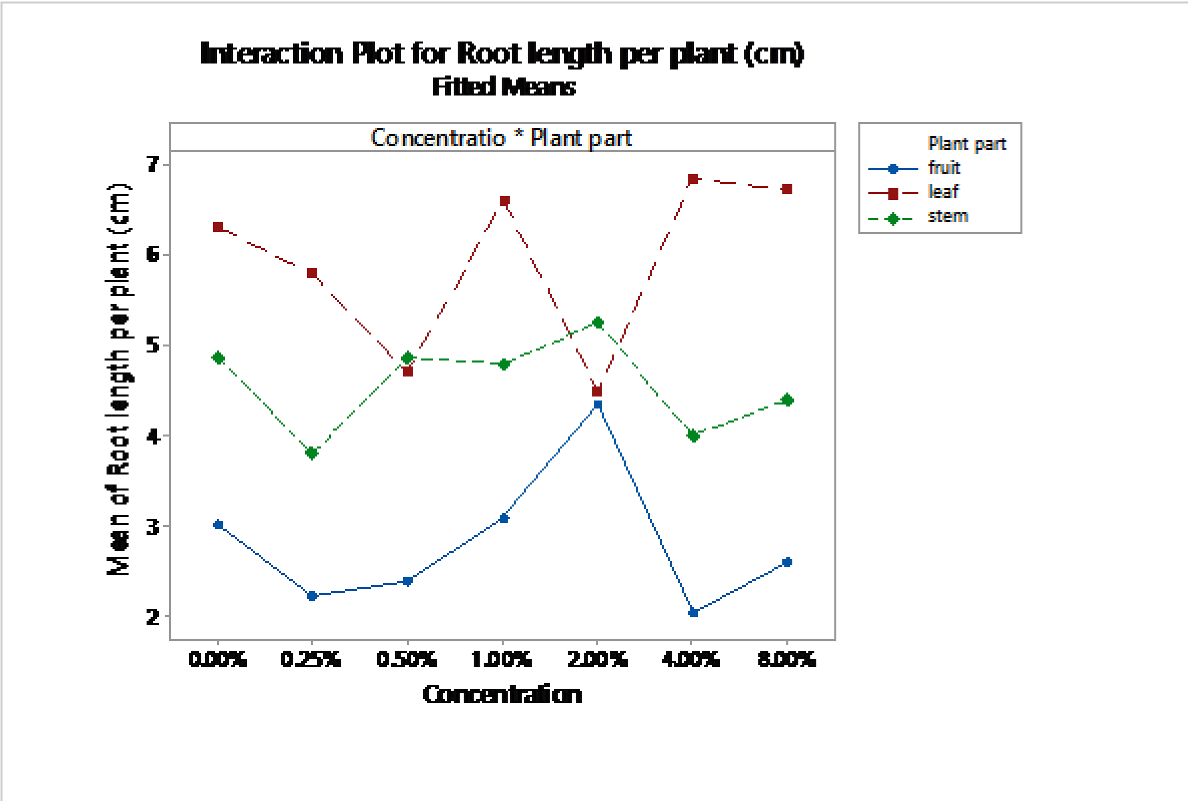

Fig. 1. Effect of aqueous extracts of *S. oleraceus* on root length of *O. punctata*.

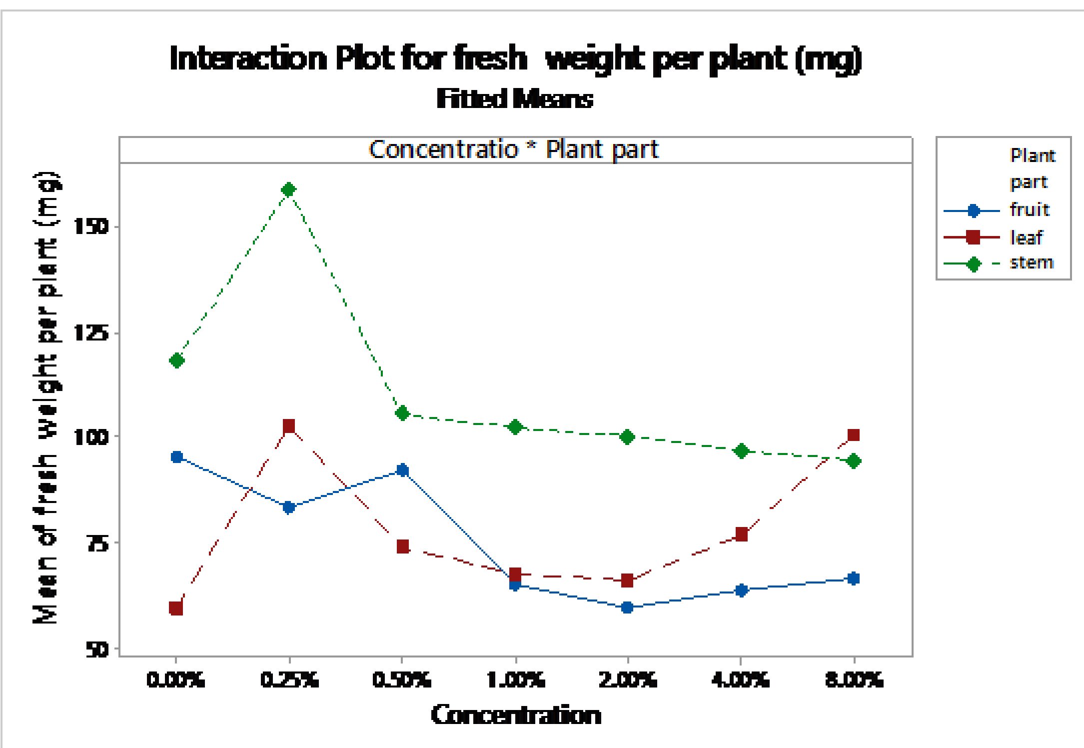

Fig. 2. Effect of aqueous extracts of *S. oleraceus* on fresh weight of *O. punctata*.

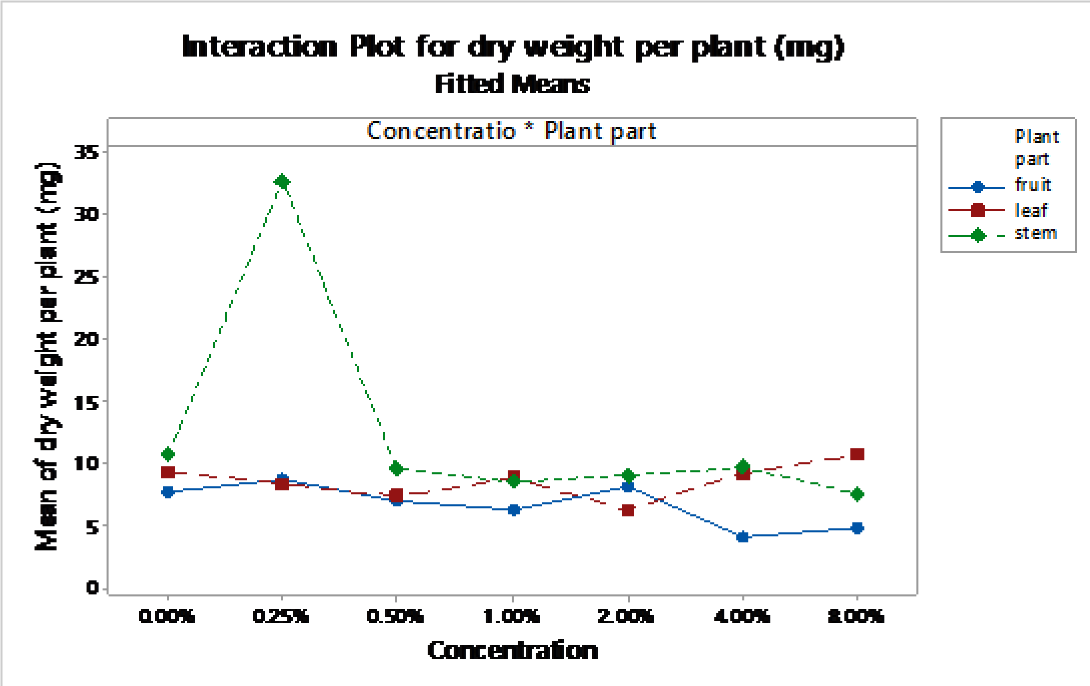

Fig. 3. Effect of aqueous extracts of *S. oleraceus* on dry weight of *O. punctata*.

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