ALLELOPATHIC POTENTIAL OF ORANGE FRUIT WASTES AS A NATURAL BIO-HERBICIDE IN CONTROLLING CANARYGRASS AND CHEESEWEEDMALLOW INFESTING COMMON BEAN PLANTS

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Abstract

This study was conducted to evaluate the bioherbicidal properties of orange peels against canary grass and cheese weed mallow and the response of common bean plants. Two successive pot experiments were conducted with twelve treatments. The first four treatments were applied by incorporating of orange peels powder with the soil surface at successive rates (10, 20, 30 and 40 g/pot) one week pre-sowing of common bean. In the other corresponding four treatments, the orange peels powder was incorporated with the soil surface at the same rates but directly at the same time with sowing of common bean seeds. Additionally, four untreated control treatments were applied for comparison. The recorded results revealed the inhibitory allelopathic effects of orange peels powder on both weeds with direct relationship between the orange peels rate and it's inhibitory effects. However, the pre-sowing treatments were more effective than the others which applied at the time of sowing. Orange peels at 40g/pot at one week pre-sowing gave the highest inhibitory effect on both weeds. This superior treatment subsequently recorded the highest growth parameters and yield traits of common bean. Estimated phenolic compounds, flavonoids and essential oils in orange peels may be responsible for this inhibitory effect.

Keywords: Allelopathy, Orange peels, Common bean, Weeds, phenolic compounds, flavonoids, essential oils

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Introduction

Common bean *(Phaseolus vulgaris*) is an annual legume crop. In Egypt, where climatic conditions are favorable for its production, edible dry seeds or unripe fruits are produced for human consumption and export. Common bean dry seeds are protein-rich and good source of zinc and iron (Burucharaet *al.* 2011).Common bean is a healthy food as its low glycemic index (Widers, 2006).Weeds area serious problem in crop production that not only decrease common bean yield but also affect negatively on its quality. However, Pynenburg *et al*. (2011) revealed that weed competition caused common bean dry seed yield reduction reached to 85%.The vegetative growth stage is the critical period at which common bean plants are sensitive to weed competition (Blackshaw, 1991).So, the scientists make their efforts to manage weeds at the limit not effect on crop production. Although the chemical herbicides application is still the predominant method of controlling weeds it causes serious problems. The Frequent and excessive use of chemical herbicides led to environmental pollution beside the resistance of weeds to these herbicides (Rao *et al.,* 2007;Mehdizadeh and Gholami 2018). The existence of these problems has urged the scientists to find safe alternative methods (Mehdizadeh and Mushtaq 2020). The application of allelopathy in controlling weeds is as safe alternative challenge to these problems (Jabran *et al.* 2015). Allelopathy is a phenomenon naturally occurs between plants by releasing chemical compounds called allelochemicals (Shahrokhi *et al.* 2011). Allelochemicals have a positive or negative effect on the other plants (including microorganisms) in direct or indirect manner(Krenchinski *et al.* 2017). Allelo chemicals reduce the attack of weeds and other pests by inhibiting their germination and development. Additionally, allelo chemicals have a great impact on the soil by improving its physical and chemical characteristics as well as regulation of the microbial community. Allelo chemicals perform all these strategies aiming to reduce weeds competition at the limit where they do not affect the yield (Pedrol *et al.* 2006; Grisi *et al*. 2012). Waris *et al*. (2016) reported that allelopathy is a viable technique and can be successfully used in agriculture.

Egypt is one of largest orange (*Citrus sinensis* L.) producing countries all over the world. Orange peels are the major waste part of orange juice process. Orange peels contain a large number of phytochemical active compounds such as flavonoids which are responsible for the color of orange fruit (Davies, 2000). Nishimura *et al.* 1969; Nogata *et al.*1994 identified and quantified flavonoids in orange peels. Although Mode of action by which flavonoids contribute in allelopathy is still unknown, it can be applied as allelopathic active compounds in controlling weeds (Mierziak *et al.* 2014). Flavonoids may inhibit cell growth, disturb ATP production or hinder the auxins functions (Berhow and Vaughn, 1999). Orange peels are frequently rich with essential oils. Essential oils have been approved as germination and growth inhibitor to other plant species (Duke *et al.* 2002; El-Rokiek *et al.* 2018). Orange peels essential oils are highly active bio herbicides (Sharma and Tripathi, 2006; Ali and Çelik,2007; Tsai, 2008; Ribeiro and Lima, 2012; El Sawi *et al.* 2019). The objectives of this study were:

1- To determine the allelopathic response of common bean plant and two associated weeds canary grass and cheese weed to orange peels in powder form
2- To estimate the most effective orange peels rates to be applied under field condition.
3- To optimize which time is most effective to add orange peels one week pre sowing or at sowing as a natural bioherbicide?

Materials and Methods

A- Preparation of plant material

Fresh orange (*Citrus sinensis*L.) fruits were purchased from the local supermarket. The peels were separated from these orange fruits, dried under shade for several days. Dried orange peels were ground separately into a fine powder using an electric mill and stored at 4 °C.

B- Experimental procedure

Two pot experiments were carried out on February during two successive spring seasons of 2018 and 2019in the greenhouse of the National Research Centre (NRC).earthen pots 30cm in diameter(0.07m^2) were fallen with equal amount of sieved sandy-loam soil. The pots were arranged in complete randomized block design with six replicates. Seeds of common bean (*Phaseolus vulgaris)*(cv. Giza 6)were obtained from Agricultural Research Centre, Egypt. In four treatments with its 6 replicates orange peels powder was incorporated with the soil surface at successive rates 10, 20, 30 and 40 g/pot. After incorporation step pots were infested same number (ten seeds) of canary grass(*Phalaris minor*) and cheese weed (*Malva parviflora*)and mixed thoroughly. One week after incorporation and sowing weeds, five seeds of common bean were sowed. The corresponding other four treatments treated with the same rates of orange peels powder. But it differed from the previous four treatments that the same number of common bean and both weeds seeds sowed directly after incorporation. Additionally, four control treatments i.e. common bean only, common bean + cheese weed, common bean + canary grass and common bean + both weeds were applied for comparison. Thinning of common bean seedlings was done after two weeks for all treatments so that three homogeneous seedlings were left per pot. All treatments were maintained under greenhouse condition and all cultural practices were applied.

C- Studied parameters

1- On weeds

Three replicates were collected from each treatment at 70 and at harvest (100 days after sowing (DAS)) and dry weight of both canary grass and cheese weed (g/pot) were recorded.

2- On common bean plants

- **Growth parameters**

In both seasons at 70 DAS, three replicates of common bean plants were collected from each treatment to determine shoot length /plant (cm), Total chlorophyll content(SPAD value) of the fourth leaf from the top was determined according to Soil Plant Analysis Department, Minolta Camera Co., Osaka Japan as reported by Minolta, 2013, number of leaves/plant, number of branches/plant and dry weight of plant (g). While, at harvest (100 DAS), shoot length /plant (cm), number of leaves/plant, number of branches/plant and biological weight of plant (g) were determined.

- Yield traits

At harvest, samples of common bean plants were taken from each treatment to determine number of pods/plant, dry weight of pods/ plant (g), pod length and seed yield/plant.

3- Chemical analysis of orange peels powder

- Determination of total phenolic and total flavonoids contents:

Total phenolic and total flavonoid contents (mg/g DW)were determined in orange peels color imetrically using Folin and Ciocalteu phenol reagent according to the method defined by Srisawat *et al.*(2010).

-**Determination of total essential oils:**

Total essential oils content in orange peels powder were determined by subjecting to hydro-distillation (HD) for 3 h using a Clevenger-type apparatus (Clevenger, 1928). The essential oil content was calculated as a relative percentage (v/w).

Statistical analysis

All data were statistically analyzed according to Gomez and Gomez (1984) and the treatment means were compared by using LSD at 5% probability.

RESULTS

1- Weed growth parameters

All weeded treatments with orange peels powder, either one week pre-sowing or at sowing, reduced the dry weight of canary grass and cheese weed compared with weedy check at 70 days after sowing (DAS) and at harvest in both seasons. It was observed that the use of orange peels powder treatment one week pre-sowing at successive rates was more effective than the use of it at sowing in controlling both weeds (Table

1 and Fig.1 (a and b)). However, each pre-sowing applied rate of orange peels was more effective than the same rate when added at sowing. So, in both seasons at 70 DAS and at harvest pre-sowing orange peels powder treatment at the rate of 40g/pot was the most effective treatment in controlling canary grass and cheese weed weeds. Orange peel powder applied at sowing at 40g/pot came in the second rank and so on. In this regard, unweeded check (common bean+ canary grass and cheese weed) recorded the highest value of dry weight of both weeds in the first and second seasons.

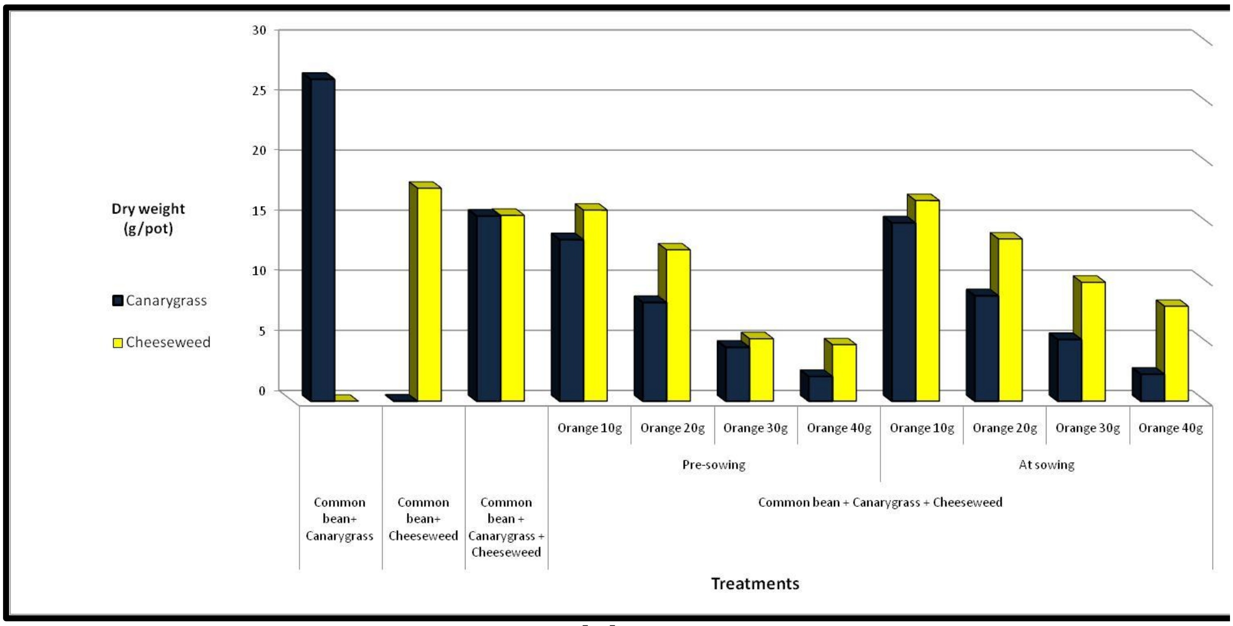
- Growth parameters:

1- Common bean

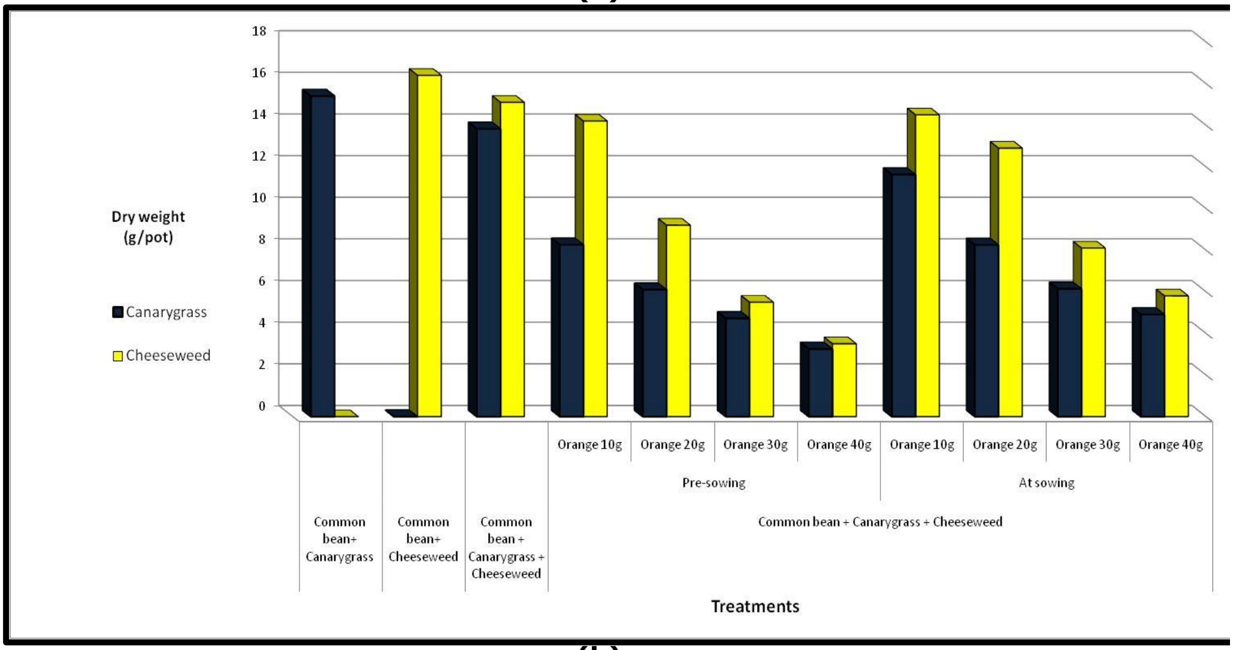
Results in Tables 2and3 reveal significant impacts of orange peels powder on plant height, SPAD value, number of leaves and branches as well as dry weight of plant in both seasons at 70 DAS and at harvest in comparison to untreated check. Incorporation of orange peels powder at 40g/pot one week pre-sowing and at sowing gave the maximum values of shoot length, number of leaves and dry weight of plant at 70 DAS and at harvest without significant differences between them. Moreover, Pre-sowing incorporation

Table 1: Effect of orange peels on the growth of canary grassandcheese weed(g / pot)in 2018 and 2019 seasons at 70DAS and at harvest

Treatment			2018						2019					
			At 70 DAS			At harvest			At 70 DAS			At harvest		
			canary grass	Cheese weed	Total	canary grass	Cheese weed	Total	Canary grass	Cheese weed	Total	Canary grass	cheese weed	Total
common bean+ canary grass			6.02	0.00	6.02	26.79	0.00	26.00	8.96	0.00	8.96	15.04	0.00	15.04
common bean+ cheese weed			0.00	7.44	7.44	0.00	17.73	17.73	0.00	6.30	6.30	0.00	16.4	16.40
common bean + canary grasscheese weed+			5.15	6.46	11.61	15.40	15.47	30.87	7.45	5.98	13.43	13.83	15.10	28.93
common bean + canary grass + cheese weed	Pre-sowing	Orange 10g	3.63	3.30	6.93	13.44	15.9	29.34	5.52	4.00	9.52	8.26	14.20	22.46
		Orange 20g	2.00	3.03	5.03	8.21	12.6	20.81	3.80	3.17	6.96	6.10	9.20	15.30
		Orange 30g	1.00	2.13	3.13	4.48	5.2	9.68	2.96	2.30	4.99	4.72	5.50	10.22
		Orange 40g	0.70	1.77	2.47	2.05	4.7	6.75	1.95	1.50	3.45	3.25	3.50	6.75
	At sowing	Orange 10g	4.34	5.10	9.44	14.84	16.7	31.54	5.71	4.10	9.81	11.63	14.50	26.13
		Orange 20g	2.43	3.83	6.26	8.77	13.5	22.27	4.38	3.33	7.71	8.25	12.90	21.15
		Orange 30g	1.57	2.96	4.53	5.13	9.90	15.03	3.12	2.87	5.99	6.14	8.10	14.24
		Orange 40g	0.96	2.54	3.50	2.24	7.90	10.14	2.04	2.60	4.64	4.91	5.80	10.71
LSD 0.05			0.67	1.05	1.14	1.03	1.14	1.25	0.84	0.65	0.96	0.56	0.67	1.34



(a)



(b)

Fig. 1(a and b): Allelopathic effect of orange peels on canary grass and cheese weed at harvest in the first (a) and second seasons(b) .

Table 2: Effect of orange peels on the growth parameters of common bean in 2018 season at 70DAS and at harvest

Treatment			2018 season								
			At 70 DAS					At harvest			
			Shoot length (cm)	SPAD value	No. of leaves	No. of branches	Dry weight /plant (g)	Shoot length (cm)	No. of branches	No. of leaves	Biological weight /plant (g)
Common bean only			30.4	34.2	5.3	2.5	4.56	40.6	2.0	8.3	8.5
Common bean+ canary grass			26.6	33.2	4.7	2.0	2.26	39.3	1.9	7.4	5.8
Common bean+ cheese weed			28.8	33.4	5.2	2.1	4.43	40.5	1.8	7.6	6.4
Common bean + canary grass +cheese weed			22.4	30.4	3.9	2.0	2.18	39.0	1.8	6.4	4.8
Common bean + canary grass +cheese weed	Pre-sowing	Orange 10g	36.2	36.2	6.3	2.2	5.10	46.9	2.3	7.7	10.9
		Orange 20g	37.1	37.1	6.2	2.3	5.33	51.1	2.3	10.1	13.3
		Orange 30g	38.4	38.4	6.3	2.3	5.63	53.8	2.7	10.8	16.5
		Orange 40g	39.6	39.6	6.3	2.6	6.20	58.9	3.0	11.1	19.4
	At sowing	Orange 10g	34.6	34.6	5.3	2.3	5.05	46.6	2.0	7.6	9.3
		Orange 20g	35.1	35.1	5.3	2.3	5.24	49.0	2.3	8.2	11.0
		Orange 30g	38.4	38.4	7.2	2.3	5.62	52.0	2.7	10.1	12.7
		Orange 40g	38.5	38.5	5.3	2.5	5.93	57.8	2.7	10.7	15.2
LSD 0.05			1.69	2.26	1.21	NS	0.63	3.53	0.70	0.71	1.77

*NS=non-significant

of orange peels positively promotes growth parameters of common bean than its incorporation at sowing. This stimulatory response was induced by increasing of orange peels rates (Fig. 2). Statistical analysis of recorded results indicated that SPAD value significantly increased in the first season but this increase was not significant in the second season. Number of branches parameter not significantly affected

along two seasons. of orange peels positively promotes growth parameters of common bean than its incorporation at sowing. This stimulatory response was induced by increasing of orange peels rates (Fig. 2). Statistical analysis of recorded results indicated that SPAD value significantly increased in the first season but this increase was not significant in the second season. Number of branches parameter not significantly affected along two seasons.

Table-3. Effect of orange peels on the growth parameters of common bean in 2019 season at 70DAS and at harvest

Treatment			2019 season								
			At 70 DAS					At harvest			
			Shoot length(cm)	SPAD value	No. of leaves	No. of branches	Dry weight /plant (g)	Shoot length (cm)	No. of branches	No. of leaves	Biological weight /plant (g)
Common bean only			32.0	36.0	5.0	2.3	2.73	42.9	2.7	7.0	7.47
Common bean+ canary grass			29.4	34.0	4.6	2.0	2.33	39.0	2.0	6.4	6.00
Common bean+ cheese weed			31.5	35.6	4.7	2.0	2.63	41.8	2.0	6.6	6.70
Common bean + canary grass +cheese weed			24.9	32.4	4.3	2.0	2.00	38.6	2.0	6.0	5.37
Common bean + canary grass +cheese weed	Pre-sowing	Orange 10g	38.8	36.4	5.7	2.3	4.39	47.2	3.1	7.7	13.14
		Orange 20g	38.3	37.3	6.0	2.4	4.38	53.7	3.3	8.1	17.52
		Orange 30g	38.7	37.9	6.2	2.4	5.13	60.5	3.3	8.8	19.47
		Orange 40g	41.7	39.5	6.2	2.5	5.52	62.4	3.7	8.8	22.78
	At sowing	Orange 10g	36.9	36.1	4.7	2.1	3.97	45.8	2.9	7.5	10.39
		Orange 20g	37.7	36.4	4.7	2.2	4.32	49.3	3.0	7.6	12.67
		Orange 30g	39.3	38.1	5.0	2.3	4.99	59.4	3.3	8.0	17.24
		Orange 40g	40.3	38.6	5.0	2.4	5.30	60.8	2.9	8.6	20.56
LSD 0.05			1.69	NS	1.07	NS	0.59	5.23	0.68	1.06	1.83

*NS =non-significant

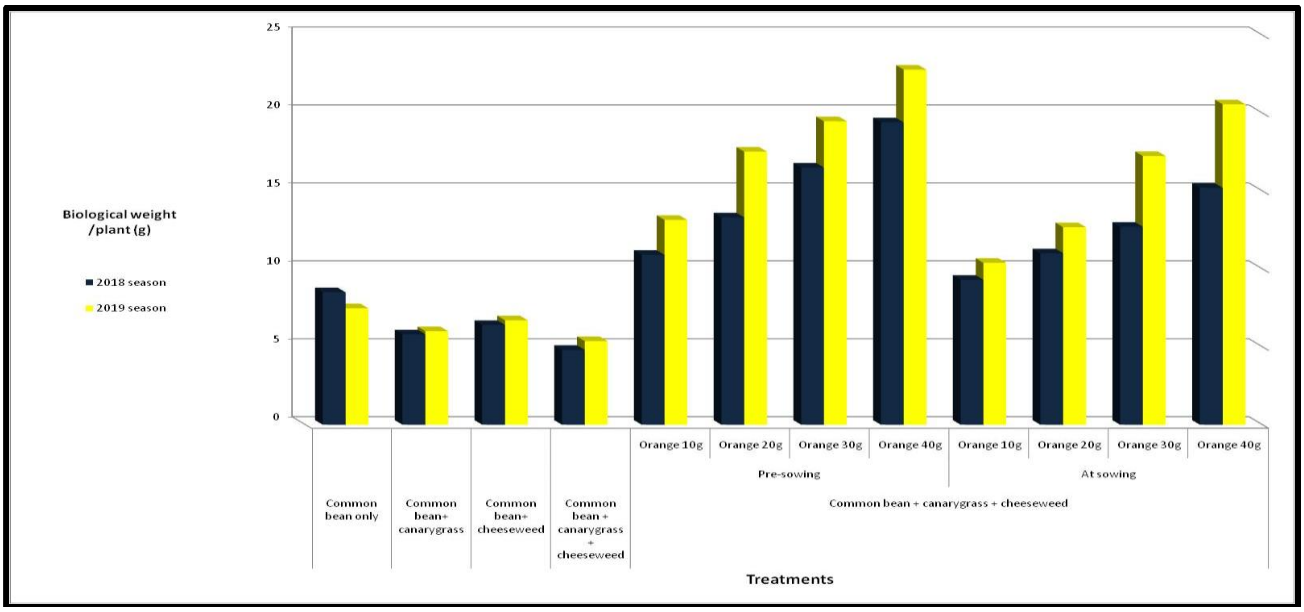


Fig. 2: Allelopathic effect of orange peels on common bean biological weight /plant (g)at harvest in 2018 and 2019 seasons

Common bean Crop Yield traits

Incorporation of orange peels powder at sequenced rates significantly increased number of pods/plant, dry weight of pods/plant, pod length and yield/ plant as compared to the untreated check (Table 4andFig. 3). However, pre-sowing incorporation of orange peels at successive rates was more effective than its incorporation at sowing as compared to untreated check. The recorded results ensured a direct relationship between the increase in rate of orange peels and the development of yield traits. Using of orange peels powder at 40g/pot one week pre-sowing as well as at sowing were the superior treatments in promoting the development of yield traits with no significant difference between them in both seasons. In contrast, the minimum values of pea yield traits were recorded with untreated check.

Table 4: Effect of orange peels on the yield traits of common beanin 2018 and 2019 seasons

Treatment			2018				2019			
			No. of pods/ plant	Dry weight of pods /plant (g)	Pod length (cm)	Yield / plant (g)	No. of pods/ plant	Dry weight of pods /plant	Pod length (cm)	Yield / plant (g)
Common bean only			4.4	2.93	5.50	3.3	4.6	3.00	5.40	3.2
Common bean+ canary grass			2.3	2.20	4.50	2.3	2.8	2.03	4.37	2.4
Common bean+ cheese weed			2.7	2.63	4.57	2.5	3.7	2.30	4.73	2.7
Common bean + canary grass +cheese weed			2.0	1.80	4.33	2.0	2.1	1.87	4.33	2.2
Common bean + canary grass +cheese weed	Pre-sowing	Orange 10g	4.4	4.37	6.67	4.3	3.9	3.53	6.13	4.1
		Orange 20g	6.1	6.10	7.10	5.3	5.7	4.57	6.73	5.1
		Orange 30g	7.7	6.80	7.53	6.6	6.9	6.00	7.40	6.5
		Orange 40g	8.6	7.37	8.10	7.8	7.7	7.23	7.87	8.3
	At sowing	Orange 10g	3.7	4.17	5.67	3.7	3.4	3.40	5.93	4.2
		Orange 20g	5.3	5.90	6.67	4.8	5.5	4.53	6.63	5.1
		Orange 30g	7.0	6.73	7.33	6.4	6.5	5.83	7.30	6.9
		Orange 40g	8.0	7.00	7.87	7.6	7.5	6.30	7.67	7.1
LSD 0.05			**	**	**	**	**	**	**	**
			1.35	0.49	1.50	1.69	0.77	0.56	0.68	1.69

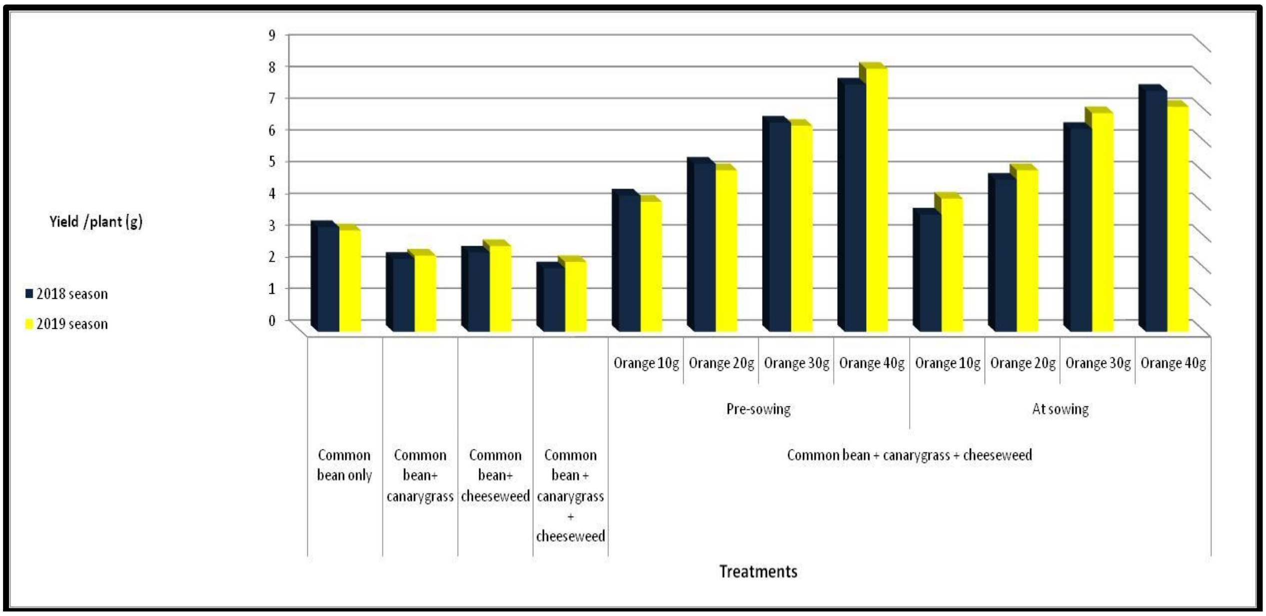


Fig.3: Allelopathic effect of orange peels on common bean yield /plant (g) at harvest in 2018 and 2019 seasons

2- Chemical analysis of orange peels powder

Table 5 show quantitatively chemical analysis of orange peels searching on allelo chemicals may be responsible for the inhibitory allelopathic effect on weeds. Total Phenolic (1.36%) and flavonoid (0.017%) contents as well as essential oils (0.64%) were already found in abundant amount. More chemical analysis is required to fractionate and identify the exactly active compound and its mode of action on plant.

Table (5): Quantitative chemical analysis of orange peels powder

Chemical analysis of orange peels	
Phenolic contents (mg/g DW)	13.6
Flavonoid content (mg/g DW)	0.0017
Essential oils (ml/ 100 g DW)	0.64

DISCUSSION

This study aimed to apply a safe allelopathic phenomenon to control weeds as alternative to the harmful chemical herbicides. The recorded results in Table (1) and Fig (1)ensured the allelopathic inhibitory effect of orange peels powder on weeds under investigation (canary grass and cheese weed) along two seasons. The inhibitory allelopathic effect of orange peels increased by increasing the rate of incorporated orange peels in the soil. The allelopathic inhibitory effect ensured the presence of bioactive compounds which translocated from the peels tissues to the soil. The previous report of Goren and Goldschmidt, 1970confirmed that mature orange fruits contain abundant amount of growth promoters. Also, Goldschmidt et al*.* 1972 found that abscisic acid-like growth inhibitors were formed in a huge amount rapidly in orange peels after harvest. Similar findings ensured the presence of essential oils Ribeiro and Lima, 2012; El Sawi et al*.* 2019and fatty acids Nuneset al*.* 2015 in orange residues that has inhibitory allelopathic effect and have been suggested to be applied in a safe weed management strategy. An allelopathic phenomenon in other citrus plants has been reported by many scientists. However, AlSaadawi *et al.*(1985)identified some inhibitory allelopathic compounds in sour orange leaves on the growth of *Amaranthus retroflexus*. Likewise, Mansour*et al.*(2014)confirmed that sour orange peels inhibit the growth parameters of the same weed. Extract of yuzu fruit peels strongly suppressed lettuce seeds(Fujihara and Shimizu, 2003). Kato-Noguchi and Tanaka 2003(2004) found that ABA-GE was the main growth inhibitors in yuzu fruit. It is noteworthy that incorporation of orange peels one week pre-sowing common bean was more effective in controlling weeds than the corresponding same treatments but orange peels incorporated at sowing. This may be discussed as one week earlier in mixing orange peels powder gave enough time to allelopathic compounds to be dissolved and affect negatively on weeds (already present in soil) without any effect on absent common bean seeds. Moreover, the active orange peels allelopathic compounds easily soluble in irrigation water and readily diffusible in the soil, the same note was approved by Fujihara and Shimizu 2003on yuzu peels.

As orange peels powder was effective in controlling weeds, the competition consequently was limited on environmental parameters which became available to promote common bean development as compared to untreated check (Table 2,3 and Figs 2,3). Also, may be related to the allelopathic stimulatory effect of orange peels. These conclusions are in accordance with those recorded by Jabran *et al.,* 2015; El-Wakeel *et al.*, 2019. Stimulation of common growth parameters reflected in turn on yield traits (Table 4 and Fig. 3). It was noticed that common bean yield traits positively affected by the applying of orange peels and this positive response increased by increasing orange peels mixed rates as limitation of weeds increased at high rates. Additionally, one week pre-sowing incorporation of orange peels gave higher growth parameters and yield traits than the incorporation of orange peels at sowing of common bean seeds. This may be related to the avoiding of inhibitory effect of orange peels by delaying common bean sowing one week after orange peels incorporation. Through this week the inhibitory effect of orange peels affected on weeds only.

Nevertheless, phenolic and flavonoid compounds as well as essential oils in orange peels have been estimated in our laboratory which may be responsible for the inhibitory effect of orange peels. Table 5 ensured the presence of abundant amount of these compounds in orange peels. Allelo chemicals such as phenolic compounds or flavonoids directly affect the physiological processes of plant, i.e. mitotic activity, nutrient uptake, permeability of cell membrane, photosynthesis, and respiration as well as enzyme activity inhibition and protein formation (Rice, 1984; Wu *et al.* 2000; Xuan *et al.,* 2004).Also, essential oils in orange peels have been reported before to have inhibitory effect on weeds (Sharma and Tripathi, 2006; Ali and Çelik2007; Tsai, 2008; Ribeiro and Lima, 2012; El Sawi *et al*., 2019). Orange peels as a waste material of food industry represent a problem to be discarded. The present study suggests that this fruit residue may be utilized as a safe and useful material for weed control. More studies should be applied on orange peels to fractionate and identify the exactly active compounds and determine its mode of action on crops and associated weeds.

CONCLUSION

Controlling weeds through allelopathy is an alternative strategy to reduce dependency on synthetic herbicides. So, this study suggests using orange peels as a weed-suppressive residue. Incorporation of orange peels one week pre-sowing at 40g/pot was the most effective treatment to control canary grass and cheese weed weeds infested common bean crop.

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