Plant Archives Volume 20 No. 2, 2020 pp. 9120-9124 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

EFFECT OF FOLIAR APPLICATION WITH YEAST EXTRACT AND METHANOL ON MORPHOLOGICAL AND YIELD CHARACTERISTICS OF FABA BEAN (*VICIA FABA* L.)

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Abstract

Pot experiment was carried out to determine the effect of separate foliar application of bread yeast (1, 2, 3 and 4%) and methanol (10, 15, 20 and 25%) on vegetative growth, leaves chlorophyll content, flower numbers, nutrient elements and crude protein content in seeds and yield components of broad bean (*Vicia faba* L.) *cv.* Super Aquadulse plants. The experiment included nine foliar spray treatments with 3 replicates in a complete randomized design. Yeast or methanol spraying caused significant increases in leaf area, shoot fresh and dry weight, photosynthetic pigments in leaves, flower numbers, seed N, P, K and crude protein, pod length and seed yields. The enhancement effects of yeast extracts were more pronounced than methanol treatments in improving growth and yield quality. In addition, the yeast treatments at 2 and 3% as well as 10 and 15 % of methanol treatment had the superiority effects in improving plant parameters.

Key words: Methanol, yeast, broad bean, foliar application

Introduction

Faba bean, is one of the oldest domesticated plants, is used for human and animal nutrition because of their seeds with high protein (about 25%) content (El-Moghazy *et al.*, 2011). The increasing of crop production is one of the most important targets of agricultural policy in several countries (Mona *et al*., 2013). In recent years, the world focused his attention to minimize the environmental pollution by reducing the use of synthetic fertilizers and chemicals in crops production. Therefore, several researchers tend to use environmentally safe organic substances and costless to encourage the yield and quality of plant. The yeast is natural source of cytokinins and has stimulatory effects on bean plants, it was suggested to participate in a beneficial role during vegetative and reproductive growths through improving flower formation and their set in some plants due to its high auxin and cytokinins content and enhancement carbohydrates accumulation (Barnett *et al*., 1990 and Amer, 2004). Improving growth, flowering and fruit set and yield of some plants by using foliar application with yeast extract was reported by Wanas (2006), Khalil and Ismael (2010), Sarhan *et al*., (2011) and Abou El-Yazied and Mady (2012) and Abaas (2013).

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Exogenous application of methanol enhanced plant biomass and yield of different C_3 crop species. Several studies have been shown that foliar application of methanol increased plant biomass and yield (Mona *et al.,* 2013). Methanol spray is introduced as a suitable method which can increase CO_2 assimilation (Hossinzadeh *et al.,* 2012). Foliar applications of methanol in various plants have been reported to improve yield and reduce stress (Ramirez *et al.,* 2006; Mirakhori *et al.,* 2010). It has been stated that foliar utilization of methanol, as a source of carbon, increases the growth and yield of different plant species (Bagheri *et al.,* 2014).

Therefore, the present investigation was designed to disclose the influence of different levels from yeast extract at 1, 2, 3, 4% and methanol at 10, 15, 20, 25 % on vegetative growth, leaves content of chlorophyll and yield attributes offaba bean plant cultivar.

Materials and Methods

This investigation was conducted during (2018-2019) growing season at the green house of Biology Department, College of Education, Salahaddin University-Erbilto determine the effect of separate foliar application

of bread yeast (1, 2, 3and 4%) and methanol (10, 15, 20 and 25%) on some vegetative growth parameters, leaves chlorophyll content, nutrient (N, P, K) content, crude protein content in seeds, yield and yield components of broad bean (*Vicia faba* L.) *cv.* Super Aquadulse plants. The experiment included nine foliar spray treatments with 3 replicates in a complete randomized design, *i.e.* the control (sprayed with distilled water), yeast extract at 1, 2, 3 and 4% (w/v), Methanol at 10, 15, 20 and 25% volumetric percentage (v/v). Methanol and bread yeast extract were applied twice as foliar spray during vegetative growth at 45 and 60 DAS using hand operated compressed air sprayer, Spray application was continued until solution drops flow from plant surface. Yeast extract prepared from commercial baking yeast dissolved in water followed by adding sugar at ratio 1:1 and kept overnight for activation and reproduction of yeast. Plant samples were taken at 2 weeks after the second spraying treatment and at beginning of flowering stage to determine some growth parameters. A random sample of 6 plants for each tested treatment (2 plants from each replicate) was assigned for investigation. Vegetative characters were recorded two weeks after the second application yeast and methanol treatments included plant height (cm), number of secondary branches plant^{-1}, number of leaves plant^{-1}, total leaf area cm^2 plant^{-1}, fresh and dry weight of shoot g plant^{-1}, Leaf area was measured by a simple and quick leaf area estimation model for faba bean (Erdoan, 2012):

LA= -1, 6923 + (L*0, 0161) + (W*0, 0929) + (0, 0062*L*W)

Where LA is leaf area (cm^2), L is leaflet length (cm), W is the leaflet width (cm).

Chlorophyll *a* and *b* were estimated by the spectrophotometric method in fresh leaves of plants as mgg^{-1} fresh weight according to the method of Arnon (1949):

Chl a = [12.7 (OD 663) -2.69 (OD 645)] × V/1000 × W

Chl b = [22.9 (OD 645) -4.68 (OD 663)] × V/1000 × W

V = volume of the extract (mL), W = weight of the fresh leaf tissue (g). No. of opened flowers plant^{-1}were counted after 10 weeks from sowing. A random sample of 6 plants for each tested treatment (2 plants from each replicate) was taken at harvest time, 12 weeks from sowing date, to investigate the characters which included pod length(cm), No. of pods plant^{-1}, No. of seeds pod^{-1}, No of seeds plant^{-1}, 100 seeds weight (g) and seeds yield (g) plant^{-1}. Percentages of N, P, K and crude protein were determined in seeds, at harvest time; samples resembling various treatments beside the seeds of control plants were finely ground. Total nitrogen was determined using the modified micro- Kjeldahl method, potassium by flame emission photometer technique and phosphorus was determined using spectrophotometer method (AOAC, 1995). Nitrogen content of seeds was multiplied by 6.25 to calculate the crude protein (Ryan and Rashid, 2001). Least significant difference (L.S.D.) at 0.05 probability levels were used for comparison means treatments (Snedecor and Cochran, 1980).

Results and discussion

Effect of yeast and methanol extract on vegetative growth parameters

Table 1 showed that, yeast at concentrations of 1, 2 and 3% were significantly (p>0.05) increased total leaf area (cm^2) plant^{-1}, as well as fresh and dry weight (g) of shoot system as compared with the water spraying control. The highest values of the mentioned parameters were 47.53(cm^2) plant^{-1}, 21.67g and 11.93g respectively, recorded at 3% yeast extract. These results partially agreed with those obtained by Wanas (2006) and El-Tohamy *et al.*, (2007), that could be due to yeast contents of macro and micro nutrients, growth regulators, sugars, vitamins (especially vitamin B) and a relatively large proportion of free amino acids and short peptides of two or three amino acids as well as long chain protein hydrolysates, it is also a natural source of cytokinins that stimulates cell proliferation and differentiation, controlling shoot and root morphogenesis and chloroplast maturation (Abou El-Yazied and Mady, 2012 and Marzauk *et al*., 2014). Wanas and El- Sherbeny *et al*., (2007*)* mentioned

Table1: Effect of yeast and methanol on some vegetative growth parameters of faba bean.

Treatment		Total leaf area (cm^{2}plant^1)	Plant length (cm)	Leaf number	Branch number	Shoot fresh weight (g)	Shoot dry weight (g)
Control		34.12	66.33	10.67	1.83	15.33	9.90
Yeast	1%	43.99	68.89	10.94	1.89	19.67	10.83
	2%	39.44	70.00	10.11	1.67	21.00	11.70
	3%	47.53	70.78	11.89	1.22	21.67	11.93
	4%	38.40	69.11	11.89	1.67	16.67	10.90
Methanol	10%	37.83	65.22	9.55	1.44	17.67	10.03
	15%	49.28	71.39	12.44	1.44	20.67	10.97
	20%	49.43	68.33	10.11	1.89	22.67	11.33
	25%	40.73	67.00	10.44	1.45	15.83	9.87
L.S.D$_p$<0.05		4.91	n.s	n.s	n.s	2.39	1.04

that enhancement effect of yeast extract might be attributed to its stimulating effect on photosynthetic pigments and improvement of photosynthesis process and enzyme activity which in turn encourage vegetative growth of broad bean. From the same table, it was found that methanol treatments at 15 and 20% were significantly increased total leaf area (cm^2) $plant^{-1}$, fresh and dry weight (g) of shoot system as compared to control and there were a significant difference between treatments. The obtained results are in agreement with those made by (Li *et al.,* 1995) on soybean. The improvement of faba bean plant growth in response to the exogenous application of methanol extract may be attributed to reduced photorespiration and thereby promoting net photosynthesis (Zebic *et al.,* 1997). Therefore, methanol spray increases crop CO_2 fixation in unit area. Today, in order to achieve this goal with C_3 plants, compounds such as methanol, ethanol, propanol, butanol and amino acids like glycine, glutamate and asparate are used (Hossinzadeh *et al.,* 2012).

Effect of yeast and methanol on the leaves chlorophyll content

Table 2 reveals that all yeast treatments were significantly increased ($p<=0.05$) leaves content of chlorophyll *a* (chl.*a*), chlorophyll *b* (chl.*b*)and total chlorophyll and the highest level were recorded when faba bean plants sprayed by (3%) yeast extract. These findings are in agreement with the results of El-Tohamy and El-Greadly (2007), Abou EL-Yazied and Mady (2012) and Kamal and Ghanem (2012) on bean plants and Mahmoud, *et al.,* (2013) on pea plants. Shalaby and El-Nady (2008) reported that the increase in photosynthetic pigments could be attributed to the role of yeast cytokinins in delaying the aging of leaves by reducing the degradation of chlorophyll and enhancing the protein and RNA synthesis. Yeast increased the release of carbon dioxide through fermentation process that effectively activates the photosynthesis and accelerates the biosynthesis of carbohydrates (Mady, 2009). The improvement of photosynthetic pigments in response to the foliar application of yeast may be attributed to bioregulators which affect the balance between photosynthesis and photorespiration in plants (Abou El-Yazied and Mady, 2012). The increase in chlorophyll contents by yeast treatment was reported by (Wanas, 2002 and Mady, 2009) and this could be due to activation of chlorophyll biosynthesis (Al. El-Shafey *et al*., 2016). As indicated in table 2, the different levels of methanol treatments were significantly increased chlorophyll *a* (chl.*a*), chlorophyll *b* (chl.*b*)and total chlorophyll. The highest value of chlorophyll *a* (1.65), chlorophyll *b* (1.22) and total chlorophyll (2.87 mg g^{-1} Fw.) were obtained by foliar applying of 15% methanol. This results partially agreed with those obtained by Hossinzadeh *et al.,* (2012) and Mona *et al.,* (2013). Ramirez *et al.,* (2006) explained that the methanol application reduced photorespiration, increased photosynthesis pigments and activity. In study reported on cotton, spraying methanol, leads to the stimulation of growth by increasing cytokinins (Hossinzadeh *et al.,* 2012). In addition, foliar application of methanol delayed senescence of leaves through its effect on ethylene production, increases photosynthetic active period and leaf area via regulation of pectin methyl esterase gene (Bagheri, 2014).

Table 2: Effect of yeast and methanol foliar application on the leaves chlorophyll content (mg/g).

Treatment		Chlorophyll a ($mg.g^{-1}$)	Chlorophyll b ($mg.g^{-1}$)	Total chlorophyll ($mg.g^{-1}$)
Control	0%	0.94	0.75	1.69
Yeast	1%	1.61	1.11	2.72
	2%	1.57	1.13	2.70
	3%	1.61	1.17	2.80
	4%	1.23	1.14	2.37
Methanol	10%	1.11	1.16	2.27
	15%	1.42	0.96	2.38
	20%	1.65	1.22	2.87
	25%	1.16	1.17	2.33
$L.S.D_p<0.05$		0.18	0.28	0.36

Table 3: Effect of yeast and methanol on some yield components of faba bean plant.

Treatment		Flower number $plant^{-1}$	length of pod (cm)	No. of pods $plant^{-1}$	No. of seed pod	No. of seed $plant^{-1}$	Weight of 100 seed (g)	Seed yield $(g)plant^{-1}$
Control		20.38	8.01	9.46	3.45	32.63	87.44	28.53
Yeast	1%	22.13	8.41	10.03	3.18	31.89	92.11	29.37
	2%	26.80	8.79	9.74	3.73	36.33	102.56	37.26
	3%	28.66	9.93	10.08	3.80	36.33	108.40	39.38
	4%	19.78	9.13	8.93	3.81	38.30	99.65	38.18
Methanol	10%	22.55	9.64	8.89	3.74	34.35	96.20	33.04
	15%	20.07	8.64	8.99	3.57	33.72	98.87	33.33
	20%	21.11	8.33	9.03	3.19	30.80	96.01	29.57
	25%	23.34	8.12	9.22	3.11	31.67	94.64	29.97
$L.S.D_p<0.05$		6.23	0.54	n.s	n.s	n.s	8.71	4.62

Effect of yeast and methanol on flower numbers and yield components

According to the results in table 3, it was observed that foliar application of yeast extract at concentration of 2 and 3% led to significant increases in flower number plant-

[1]. Yeast extract treatments were played beneficial role during reproductive growths through improving flower formation and their set in some plants due to its high auxins and cytokinins contents and its enhancement effect on carbohydrate accumulation (Mady, 2009). Foliar application of yeast at 2, 3, 4% and methanol at 10 and 15% resulted in the significant increases in yield and its components (*i.e.*, pod length, 100-seeds weight and seed yieldplant^{-1}) compared to control plants. The maximum values of yield and its components were obtained as a result of foliar spray with yeast extract at 3%. The present results are in accordance with those reported by (Wanas, 2006;Abou El-Yazied and Mady, 2012;Hossinzadeh *et al.*, 2012; Mahmoud *et al.*, 2013; El-Ramady, 2014; Al. El-Shafey *et al.*, 2016).

Improvement seed yield of broad bean plants by yeast extract treatments due to increasing flower formation, pod lengthas well as increasing their ability to accumulate more bio constituents in seeds. Hayat (2007) indicated that the positive effect of yeast treatment may be due to that yeast provided plants with essential nutrients elements required for protein formation. El-Tohamy and El-Greadly (2007) revealed that yeast treatments improved pods quality of snap beans plants *(Phaseouls vulgaris)*. Mona *et al.*, (2013*)* mentioned that foliar application of methanol increased soybean growth and yield by enhancing photosynthetic capacity and internal CO_2 concentrations.

Methanol at concentrations of 20-30% caused significant yield increases of soybeans and small bean(Zbiec and Karczmarczyk, 1997).

Effect of yeast and methanol extract on seed N, P, K and protein content

As shown in table 4, foliar application of dry yeast at concentration of 2 and 3% resulted in significant increases in N, P and protein content of seeds. The highest values of mentioned nutrients were recorded by using dry yeast at a rate of 3%. However, the contents of K in dry seed tissues did not statistically different between treatments and control under both yeast and methanol treatments. The results agree with the findings of Abou EL-Yazied and Mady (2011 and 2012), Kamal and Ghanem (2012) and Mahmoud *et al.*, (2013). This could be explained on the basis that yeast treatment increased metabolic processes such as protein and carbohydrates synthesis parallel with the increased content of phytochromes. In this respect, El-Desouky *et al.*, (1998) and Wanas (2006) reported the stimulatory effects of yeast on protein synthesis. Marzauk *et al.*, (2014) also found that foliar application of yeast resulted in an increase in broad bean seeds content of nitrogen and protein percentage. As regarding to table 4 that foliar application of 15 and 20%methanol solution significantly increased the N and protein content compared to control plants, the highest values of nitrogen and protein contents were obtained from methanol solution at level of 15%. However, that may be due to the positive effect of methanol on root growth which consequently increased nitrate absorption.

Table 4: Effect of yeast and methanol on some nutrient content of faba bean seeds.

Treatment		N (mg g^{-1})	Protein (mg g^{-1})	P (mg g^{-1})	K (mg g^{-1})
Control		4.01	25.07	3.59	9.82
Yeast	1%	4.18	26.18	3.68	10.03
	2%	4.35	27.22	4.79	10.17
	3%	4.53	28.35	5.65	9.59
	4%	4.02	25.11	4.30	10.85
Methanol	10%	4.10	25.67	3.51	10.43
	15%	4.39	27.47	3.64	9.56
	20%	4.34	27.14	4.02	10.26
	25%	4.03	25.23	3.97	9.78
L.S.D$_p$<0.05		0.32	2.01	1.14	n.s.

Conclusion

Foliar application of yeast and methanol improved vegetative growth, leaves chlorophylls, yield components and seed nutrients. Yeast extracts were more powerful than methanol.

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