Journal of Medicinal Plants and By-products (2012) 1: 61-65

Original Article

Effects of Nitrogen and Pre-Harvest Desiccation on Seed Yield and Oil Quality of Evening Primrose (*Oenothera biennis* L.)

Azim Ghasemnezhad[1*] and Bernd Honermeier[2]

[1]*Department of Horticulture, Agricultural Science and Natural Resource University of Gorgan, Gorgan, Iran*
[2]*Institute of Plant Production and Plant Breeding, Justus Leibig University of Giessen, Giessen, Germany*

Article history: Received: 20 February 2012 /Accepted in revised form: 29 July 2012

Abstract

Disadvantages such as indeterminate inflorescence and high seed shattering during ripening are significant restrictions to introduce evening primrose as a commercial medicinal plant. Following to the last works, a pot experiment was conducted to clarify the influence of defoliation before harvest on the seed yield, oil and GLA (gamma-linolenic acid) percentage of evening primrose growing under nitrogen deficit. Desiccation showed no significant influence on seed yield and yield components. However, the percentage of GLA of the desiccated plants was significantly higher than that of non desiccated plants. Nitrogen did not have significant influence on GLA content, but the content of this fatty acid tending to increase with increase in the nitrogen concentration. Based on the results, it can be concluded that desiccation as a harvest method increases the oil quality with increasing the GLA percentage. Evening primrose showed a positive response to N fertilizer. Further investigation in this area seems to be necessary.

Key words*:* Evening primrose, Desiccation, Harvest time, GLA, *Oenothera biennis*, Seed

Introduction

In recent years evening primrose (*Oenothera biennis* L.) has made the transition from being a wild flower to an established agricultural crop [7, 18, 19, 25]. The seed of evening primrose is characterized by oil percentage of 25-30 % with approximately 7-10 % of γ-linolenic acid (GLA) [1, 3]. Among other sources of GLA, evening primrose oil appears to have the most biologically active form of GLA [19, 23, 28]. Diseases such as rheumatic arthritis, atopic eczema, cardiovascular disease, breast pain and premenstrual syndrome could be influenced by γ-linolenic acid [4, 13, 14, 16]. Although evening primrose has a good potential to become a commercial agricultural crop for the production of GLA, some disadvantages, such as indeterminate inflorescence, high seed shattering during ripeness and a long life cycle (biennial plant) could present significant impediments. Despite all attempts to eliminate the seed-shattering characteristic, it is still a major problem in the production of evening primrose [25]. Although there are some published information about the effects of cultivation treatments on seed yield and seed oil percentage of common evening primrose [3, 6, 7, 8, 9, 17, 20, 24, 29], the information about the effect of pre-harvest defoliation on seed yield an quality of evening primrose is negligible [10]. Defoliation (desiccation) is a standard practice in the harvest of many combinable crops [11, 26]. Defoliation is used as a means of acceleration of ripening process or removing weeds and other green materials. Seed water content, seed size, seed weight, seed protein, oil and starch content are the most important parameters that are influenced by pre-harvest defoliation [11, 27]. The effect of defoliation can be modified by cultivation methods like nitrogen application and harvest time because of their influence on the plant development. Before this study was carried out, there was no information about the interaction of cultivation techniques like nitrogen application with pre- harvest defoliation of evening primrose. Following to the last works [9, 10] the experiment was conducted to clarify the effect of pre-harvest defoliation on the seed yield, seed yield component, oil percentage and fatty acid composition of spring–sown evening primrose growing under different nitrogen supplementations.

Materials and Methods

*****Corresponding author: Department of Horticulture, Agricultural Science and Natural Resource University of Gorgan, Gorgan, Iran
E-mail Address: Azim.ghasemnezhad@agrar.uni-giessen.de

During the spring 2007 pot experiment was conducted with *Oenothera biennis* L. cv. "Anothera" at the research station of Justus Liebig University in Rauischholzhausen. In present experiment, 24 small (volume = 6.2 L) Mitscherlich pots were filled with 6 kg soil consisting two parts of pure sand and one part of local soil (4000 g sand: 2000 g soil). The soil texture was loam and pH 6.5, 31.6 mg/100 K_2O and 39.13 mg/100 g P_2O_5. Since the local soil was collected from a potato field immediately after harvest in autumn, the mineral nitrogen content was very low and not detectable. During soil preparation mineral fertilizers were added at basal levels: 0.3 g/pot phosphorus ($Ca(H_2PO_4)_2 \cdot H_2O$), 1.5 g/pot potassium (K_2SO_4), 0.4 g magnesium ($MgSO_4 \cdot 7H_2O$) and $CaCO_3$ (0.3 g/pot). The plants were grown under field conditions, but in a cage which protected against birds. During the growing period, the temperature sums were calculated by adding daily air temperatures above 5 ºC. The experimental pots were randomized in complete block design with six replications. The pots were treated with two levels of nitrogen (0.3 and 0.6 g N/pot) and harvest conventionally or defoliated using herbicide (Diquat 1.5l/ha). Different parameters such as seed yield (seed yield was calculated at the basis of 91 % DM), number of side shoot per plant, number of capsule per side shoot, number of capsule per main stem, number of capsule per pot and thousand seed weight were measured before laboratory analysis. In lab, oil and raw protein percentage as well as fatty acid composition of seed samples were analyzed. The oil percentage was determined according to Soxhlet [1, 3]. The fatty acid composition was analyzed by Varian CP 3800 gas chromatography with a modified method of Court *et al*. [3]. The percentage of nitrogen of seeds was measured using CHNS elemental analyzer EA 1110 and raw protein percentage was calculated by the percentage of N × 6.25. The statistical analysis was done using the SPSS software version 12.1. Least significant differences (LSD) at α = 5% were computed for measurements with F value <0.05.

Results and Discussion

Seed yield and Yield components

In this experiment the difference of seed yield between desiccated and non desiccated plants was not significant, but in comparison with non desiccated plants the seed yield of desiccated plants tended to reduce. It can be concluded that desiccation indirectly influence the number of capsules in different parts of the plant. For instance, defoliated plants are dryer than non defoliated plants thus the possibility of capsules shattering could increase during harvest. Contrary to that the possibility of capsules detaching from green plants (non defoliated plant) is more difficult. Both cases can significantly influence the number of capsules in different parts of plants and total capsules per pot in which result lower seed yield of desiccated plants than those of non- desiccated. Table 1 shows that seed yield (SY) of evening primrose was strongly influenced by different levels of nitrogen. The pots which were fed with 0.3 g N produced 8.8 g seed. Contrary to that, 14.8 g seed/pot was produced by pots which contained 0.6 g N. A similar result was reported by Sekeroglu *et al*. (2006) in evening primrose, El-Hafid, *et al*. 2002, in borage and Hocking *et al*. [12] in canola and Indian mustard, who showed that at certain concentrations of nitrogen, seed yield of these plants was increased. The positive effect of nitrogen on SY is probably as a result of new branches (new capsules) being formed due to increase rate of plant growth and growing period. An adequate application of nitrogen led to rapid leaf growth in the crop and enabling them to intercept more solar radiation resulting in an increasing photosynthesis. This is manifested in the plant producing more pods which is the reason behind the increase seed yield. The number of side shoots per pot (SSP), number of capsules per main stem (CMS), number of capsules per side shoots (CSS) and number of capsules per pot (CP) were measured as yield components. As presented in table 1, all of these measurements were significantly influenced by different levels of nitrogen. The highest SSP was produced by pots which were fed with 0.6 g N/pot (16 SSP). Contrary to that, 8.1 SSP were produced by pots which contained 0.3 g N/pot. When the nitrogen concentration was increased from 0.3 g to 0.6 g, the number of CMS increased from 82.0 to 93.4. The increase in the number of capsules per side shoot when pots were supplied with higher levels of nitrogen (0.6 g N/pot) was much stronger than that of low nitrogen concentration (18.9 and 59.4 CSS in pots which contained 0.3 and 0.6 g N/pot, respectively). Nitrogen improve the vegetative growth of plant, increasing the number of side shot supports more capsules production and total seed yield. This observation is in confirmation with many investigations with other oil crops. For instance Cheema *et al* [2] showed that increasing the amount of N fertilizer increased the number of pods in Canola.

Between defoliated and non defoliated plants no significant difference on seed yield and yield components was observed. The reason why no interaction between treatments on seed yield components observed is not clear. Seed oil content was not significantly influenced by used treatments in this experiment. Protein percentage was significantly influenced by different levels of nitrogen. The pots contained 0.6 g N produced 12.8% protein/100 g seed. In contrast to that 12.2% protein was produced in the pots which were supplied with 0.3 g N (table 2). The small difference between levels of nitrogen in this experiment could be a reason why oil percentage was not influenced by nitrogen. The climatic conditions might influence this effect. Hocking *et al*. [12] showed that the oil percentage of canola and

Indian mustard which were significantly influenced by nitrogen was not affected in substitute experiments. They concluded that climatic conditions and soil droughness could be the reasons of this difference. High N leaching from root medium because of high precipitation during capsules formation and seed filling during experiment, might be another reason why oil percentage was not influenced by N application. Interaction effect of treatments on protein content although no significant difference between desiccated and non desiccated plant, plants which were feed with 0.6 g N/pot produced higher protein content than those of plants received 0.3 g N/pot. No relationship between oil and protein percentage of evening primrose was observed in this experiment. Our observation is not in confirmation with the results of Reiner 1989 in evening primrose, and Zhao, *et al*. [30]; Hocking [12]; Cheema *et al*. [2] in the other oilseed crops who showed that reduction of oil percentage of oil seed crops with increasing N fertilizer reflects the inverse relationship between oil concentration and protein percentage.

Effects of different treatments on main fatty acids of *Oenothera biennis* seeds were studied in this experiment. Palmitic acid (PA) was not influenced by all treatments. Statistical analysis showed that there is an interaction between nitrogen and harvest method. Since nitrogen is a plant grows stimulator and increases the photosynthetic centres of plant by increasing the number of leaves and its life period. And desiccation as a pre harvest treatment increase the number of matured seeds per plant it was expected that the interaction of them has influence on seed oil fatty acid composition. With 0.3g N/pot defoliated plants contained higher PA percentage than conventionally harvested plants. Contrary effect was found with 0.6 g N/pot. During the first and latest harvest times defoliated plants produced the lowest PA percentage.

The percentage of oleic acid (OA) was significantly affected by different levels of nitrogen (Table 2). The highest amount of OA was produced by pots which contained 0.3 g nitrogen (11.4 and 10.4 % in the pots which contained 0.3 and 0.6 g N/pot, respectively). The percentage of linoleic acid (LA) was significantly influenced by nitrogen and harvest method in this experiment (table 2). The pots which were supplied by 0.6 g N produced the highest LA percentage (71.6% and 72.2% in the pots 0.3 g and 0.6 g N, respectively). Conventionally harvested plants produced OA higher than that of defoliated plants (Table 2). Between used treatments no interaction on LA was observed in this experiment. γ-linolenic acid (GLA), the most important fatty acid of evening primrose, was strongly influenced by harvest method. The defoliated plants tended to produce higher (8.2%) percentage of GLA than those of conventionally harvested plants (7.8%). Although there was no main effect of N on the GLA percentage, the two times interactions of nitrogen and harvest method on GLA percentage was observed. In the pots which were supplied with 0.3 g N no differences in the GLA percentage between defoliated and conventionally harvested plants was observed. Contrary to that the pots which contained 0.6 g N, defoliated plants produced the highest GLA percentage. In accordance with chronology of fatty acids synthesis, there is a negative correlation between the concentration of total monounsaturated fatty acids and polyunsaturated fatty acids in vegetable oils (22). As linoleic acid (LA) and γ-linolenic acid (GLA) positively increased with adding the N concentration it can be supposed that the increases of total polyunsaturated fatty acids might be related to the reduction of OA percentage. It means that N application indirectly reduced the OA percentage which leads to increase the percentage of LA and GLA. The gradual increase of OA in this experiment is explained by longer plant growth period of late harvest time as compare to those of earlier harvest times. The longer the growth period, the higher unsaturated fatty acid content and vice versa. With increasing concentration of nitrogen an increase in LA percentage was observed in this experiment. These observations do not conform to Reiner et al. (1989) who showed that the plants which received the lowest N concentration produced the highest LA percentage. Sekeroglu et al. [21] reported that N application did not have a clear influence on the LA percentage of Oenothera biennis cultivated under dry land conditions but in certain concentration of N the LA percentage tended to increase. An adequate nitrogen supply not only encourages leaf development, it can materially assist in retaining leaves in active photosynthesis. Thus high photosynthetic activity can accelerate capsules and seeds maturity. As the highest polyunsaturated fatty acid accumulation is expected in full ripened seed it can be a reason why nitrogen tends to increase the LA percentage. The plants which were defoliated before harvest produced lower LA percentage than those of conventionally harvested plants. Increasing in the percentage of GLA in defoliated plants might be a reason why LA in these plants is lower than conventionally harvested plants. The percentage of GLA was not significantly influenced by N concentration. These observations is in confirmation with Sekeroglu et al. [21] who reported that GLA percentage reduced by applying N fertilizer in different field conditions. El-Hafid et al. [5] showed that the percentage of GLA in borage (*Borago officinalis*) was not significantly influenced by N fertilizer. It can be concluded that GLA percentage in evening primrose seed oil is related to concentrations of other fatty acids and interaction between different agronomic factors and environmental conditions. Under high levels of nitrogen the GLA percentage reached the highest amount during the early and late

harvests, respectively. Due to higher growth rate and longer growth period of plants which were supplied

Table 1. The effect of nitrogen fertilization and pre-harvest desiccation on seed yield and yield components of *Oenothera biennis* L.

NC	HM	SY (dt/ha)	SSP	CMS	CSS	CP	TSW (g)
0.3 g	nd	9.1	8.6	82.3	21.6	103.8	0.37
	d	8.5	7.7	81.7	16.3	97.9	0.37
0.6 g	nd	15.0	16.3	94.9	58.0	152.9	0.38
	d	14.6	15.7	91.8	60.8	152.6	0.37
0.3 g		8.8	8.1	82.0	18.9	100.9	0.37
0.6 g		14.8	16.0	93.4	59.4	152.8	0.38
	nd	12.0	12.5	88.6	39.8	128.4	0.38
	d	11.6	11.7	86.8	38.5	125.3	0.37
NC	p-value	0.00	0.00	0.00	0.00	0.00	0.427
HM		0.164	0.362	0.501	0.790	0.508	0.455
NC×HM		0.675	0.855	0.646	0.406	0.554	0.646
NC	$LSD_{5\%}$	0.71	1.8	5.5	9.8	9.6	ns
HM		ns	ns	ns	ns	ns	ns
NC×HM		ns	ns	ns	ns	ns	ns

NC: Nitrogen concentration, HM: harvest method; nd: conventional harvest, d: defoliation by herbicide, SY: Seed yield, SSP: Side shoots per pot, CMS: Capsules per main stem, CSS: Capsules per side stem, CP: Capsules per pot, TSW: Thousand seed weight

Table 2. The effect of nitrogen and pre-harvest desiccation on evening primrose seed oil and seed protein percentage and the fatty acid composition of *Oenothera biennis* L.

NC	HM	SO (%)	SP (%)	PA (%)	SA (%)	OA (%)	LA (%)	GLA (%)
0.3 g	nd	29.9	11.9	7.3	1.6	11.1	71.8	7.9
	d	29.2	12.4	7.4	1.7	11.7	71.3	7.8
0.6 g	nd	29.4	13.0	7.5	1.9	10.4	72.4	7.7
d	29.7	12.6	7.1	1.6	10.4	72.1	8.6	
0.3 g		29.5	12.2	7.3	1.6	11.4	71.6	7.9
0.6 g		29.5	12.8	7.3	1.8	10.4	72.2	8.2
	nd	29.6	12.5	7.4	1.7	10.8	72.1	7.8
	d	29.4	12.5	7.2	1.7	11.1	71.7	8.2
NC	P-value	0.976	0.00	0.840	0.00	0.00	0.00	0.06
HM		0.438	0.755	0.183	0.384	0.190	0.37	0.54
NC×HM		0.091	0.02	0.02	0.00	0.121	0.735	0.01
NC	$LSD_{5\%}$	ns	0.44	ns	0.06	0.37	0.37	ns
HM		ns	ns	ns	ns	ns	0.37	0.54
NC×HM		ns	0.46	0.32	0.08	ns	ns	0.38

NC: Nitrogen concentration; nd: without defoliation, d: defoliation by herbicide, SO: Oil percentage, SP: Protein percentage, PA; Palmitic acid, SA; Stearic acid, OA: Oleic acid, LA; Linolenic acid, GLA; γ- Linolenic acid

by high level of nitrogen than plants which received moderate level of nitrogen a longer capsules maturity took place. Thus it can be supposed that a better seed maturity leads to high GLA percentage. Under high level of nitrogen conventionally harvested plants produced higher GLA percentage than that of defoliated plants. Contrary to that the GLA percentage in defoliated plants tended to increase under nitrogen deficit. Lower GLA in defoliated plants under high level of nitrogen is not explainable by direct effect of treatments. Factors such as physiological stage of plant during defoliation and seed filling might be reasons behind this conflict. Based on the results it can be concluded that desiccation as a harvest method increases the oil quality with increasing the GLA percentage. Evening primrose showed a positive response to N fertilizer. Further investigations in this area seem to be necessary.

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