

Modeling the Influence of Proline and Nickel Rates on Growth, Yield and Quality of Genoveser Basil Plant

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Abstract: To evaluate the effect of different proline and nickel rates on growth, yield and quality traits of genoveser basil an experiment was conducted as split plot design in randomized complete block design arrangement with three replications, during the successive seasons 2011 and 2012. The factors consisted of three levels of proline (0, 50 and 100 ppm) and three levels of nickel (0, 50 and 100 ppm). Different statistical analyses such as correlation and stepwise multiple linear regression were used. Results showed that the main effects of proline and nickel rates were highly significant for all studied traits. A rise in proline and nickel rates at 50 ppm increased all studied traits. In general, it is suggested that among different proline and nickel rates, proline at 50 ppm with 50 ppm of nickel are optimum for basil plant under environmental conditions of Giza Governorate, Egypt. Polynomial regression analysis indicated that the relationship between both proline and nickel rates with genoveser basil for the studied traits could be defined by using a quadratic function. The matrix of correlation showed a positive and highly significant among all possible combinations of studied traits. Also, there were full correlation coefficients (1.00) for many relations, indicating that these traits are identical and we can use any one of them to identify the other. Stepwise method demonstrated that traits such as plant height, fresh leaves yield, fresh dry flowering tops yield in the 1st cut entered to regression model and totally justified 87.51 % and 87.30 % of the variation that existed in oil yield in 1st and 2nd cuts, respectively.

Key words: Genoveser basil • Proline • Nickel • Oil yield • Statistical models • Polynomial regression • Correlation • Stepwise regression

INTRODUCTION

Basil has been cultivated for quite a while as a medicinal plant and a culinary herb in numerous nations. It has a place with the genus *Ocimum* (Lamiaceae) which contains almost 150 species of herbs and shrubs from tropical locales of Asia, Africa, central and south America [1]. Genoveser basil (*Ocimum basilicum* L. var. *basilicum*) is a standout amongst varieties of the genus *Ocimum* that is well known by its differing qualities of essential oils. Basil leaves can be utilized fresh or dry as a spice to add fragrance and flavor to confectionary, dressings, salads, pizzas, meats and soups. Its extracts are additionally utilized as a part of the manufacturing of pharmaceutical preparations, beautifiers and scents [2].

Medicinally, basil has been utilized to treat headaches, coughs, diarrhea, constipation, worms and kidney malfunction. Fresh leaves and flowers contain

essential oil of distinctive aroma that possesses advantageous impacts e.g., antiseptic, carminative, antimicrobial, sedative, anticonvulsant, antitumor and antioxidative properties. It likewise has an extensive variety of biological activities since it has insect repellent, nematocidal, insecticidal and antifungal activities [3].

Exogenous application of agrochemicals, sorted as plant growth regulators (PGRs) has been accounted for enhancing the growth and yield of different crops [4]. In the interim Ashraf and Foolad, [5] found that proline improved growth and other morphological traits of plants (as fresh: dry weight, shoot: root weight, shoot: root length) and seed yield and also has an important role for increasing plants resistance to abiotic stress. In addition, proline counteracted the adverse effects of salt stress by stimulating growth of cells and plants, improving metabolism and reducing oxidation of membrane lipids [6-9].

Also, some authors reported that proline helps plants to recoup quickly from cellular damage brought about by abiotic stresses [10]. Proline scavenges reactive oxygen species (ROS) produced in plants. It acts as a compatible solute that adjusts the osmotic potential in the cytoplasm [11]. However, accumulation of proline in the cytoplasm reported to be accompanied by a deified in the concentrations of less compatible solutes [12].

Currently, limited information is available about the effect of exogenous proline on growth or yield of medicinal or aromatic plants. Exogenous application of proline (200-800 μM) at flowering stage of coriander was useful to enhance the yield to 93.53% in genotype and 65.44% in another one. However, the maximum seed yield (5.41 and 5.17 g plant^{-1}) of coriander was recorded in the treatment involving proline at 200 and 400 μM , respectively [13].

The maximum value of dry weight was obtained when stevia callus was treated with proline at rate of 200 ppm. In the same direction, data revealed the positive effect of proline to reduce the level of lipid peroxidation (malondialdehyde content), which used as a biomarker to measure oxidative stress in stevia callus [14].

Nickel (Ni) is a component of the enzyme urease, which earlier discovery in 1975 [15]. Many researchers demonstrated the growth responses of plants to Ni additions. The earliest investigation under field conditions was reported by Brown *et al.* [16]. They indicated that Ni deficiency has a wide range of effects on plant growth and metabolism. These include effects on plant growth, plant senescence, N metabolism and Fe uptake. Also, Graham *et al.* [17] mentioned that Ni may have a role in phytoalexin synthesis and plant disease resistance. So, low applications of Ni are known to be essential for many plant species to complete their life cycle, but higher concentrations are toxic and may cause severely reactions [18]. Therefore, Ni should be classified as a micronutrient element essential for all higher plant growth.

On parsley, Atta-Aly [19] found that low levels of Ni fertilization, (50 mg/kg soil), cause increments of leaf yield and quality without any modifications in leaf chlorophyll and Fe contents. Also, results suggested that low levels of Ni fertilization helped leaves to become safer for human consumption. Also, Helmy *et al.* [20] found that low levels of Ni fertilization (40 mg/kg soil) increased coriander leaf yield and quality.

Statistical models which used to predict basil yield under different proline and nickel rates could be helpful in the management of basil. In this study, several statistical

models made to describe the effect of proline and nickel on growth, yield and quality traits by using: i) polynomial regression analysis, which to model the expected value of a dependent variable y in terms of the value of an independent variable x. ii) Correlation between yield and quality traits is very important to select desirable genotype. iii) Stepwise regression could reduce the effect of non-important traits in regression model [21-23].

The aim of this study was to evaluate the effect of different levels of proline and nickel on growth, yield and quality traits of genoveser basil in order to 1) achieve the optimum use of resources, 2) to discover if proline may counteract the adverse effects of nickel on growth. 3) to develop statistical models to describe the sequence of tests used to model curves of such variables, using polynomial regression. Also, to estimate the best selection criteria for yield improvement in genoveser basil breeding program using correlation and stepwise regression analysis.

MATERIALS AND METHODS

Description of the Experimental Site: This investigation was carried out during the two consecutive seasons of 2011 and 2012 at the Agricultural Research and Experimental Station, Faculty of Agriculture, Cairo University, Giza, Egypt (30° 02' N latitude and 31° 13' E longitude with an altitude of 22.50 meters above sea level). The experimental area was a silt loamy soil of the following properties: sand, 25 %; silt, 54 %; loam, 21 %; organic matter, 0.39 %; total N, 1.12 % total P, 0.088 %; total K, 0.20 %; total C, 0.23 %; pH, 7.84 and EC (ds m^{-1}) 2.35. The soluble ions (meq/L) were SO_4 , 8.5; Ca^{+2} , 12.5 and HCO_3 , 2.5.

Experimental Layout and Herb Management: Seeds of genoveser basil (*Ocimum basilicum* L. var. basilicum) that obtained from Enza Zaden (Deutschland GmbH & Co. KG) were sown in seed beds during the second week of February in both seasons. The field was divided into 27 plots; each plot was 5.4 m^2 comprising of 3 rows 3 m long and 60 cm apart. After 40 days from sowing, the seedlings were transplanted into the field at 25 cm apart immediately after irrigation. During soil preparation, 150 kg/fed (It is a local area used in Egypt equal 4200 m^2 , where 1 hectare = 2.4 feddan), superphosphate (15.5 % P_2O_5) was applied. After transplanting, 200 kg ammonium nitrate (33.5 % N) and 100 kg potassium sulfate (48 % K_2O) per fed. were added. The mineral fertilization was divided into two equal portions during the growing season,

the first portion was added after one month of transplanting, while the second one was applied after two weeks from the first cut. All agricultural practices were carried out as usually recommended for basil production in Egypt.

Nickel sulphate salts (as a source of Ni) was added as foliar application at the rates of 0, 50 and 100 ppm, while proline was sprayed at levels of 0, 50 and 100 ppm. Both of them were sprayed four times during the growing season, twice in each cut. The first one was applied after one month of transplanting, while the second was sprayed in the first week of May in both seasons. Meanwhile, the third one was sprayed after one month of the first cut. The fourth one was carried out in the middle of August in both seasons. All spraying treatments were applied at the early morning and spreading agent "Masrol" was added to the foliar nutrient solution (1ml/l) to reduce surface tension. The experiment was designed as split plots with proline as main plots and Ni as subplots in three replicates.

Data Collection and Sampling: Genoveser basil plants were harvested twice during the growing season. The first harvest was done during the second week of July and the second one in the middle of October in both seasons. For each harvest, 10 plants were randomly selected for determining the growth parameters.

Representing samples of air dried herb of each replicate at each harvest in both seasons were subjected to hydro-distillation for 3 hours using Clevenger apparatus to extract and to determine essential oil percentage according to Egyptian Pharmacopoeia, [24]. Essential oil was separately dehydrated over anhydrous sodium sulphate and kept in silica vials with Teflon-sealed caps and stored at 2°C in the absence of light till GC analysis. The percentage of extracted essential oil was determined and recorded on the basis of oil volume to herb dry weight (ml/100g dry herb). Essential oil yield (L/fed.) was also calculated and recorded.

The essential oil constituents were analyzed and determined in the oil samples of the first cut in the second season. The dehydrated oil of each treatment was subsequently analyzed using a Gas Liquid Chromatography-mass Spectrometer (GC-MS) to evaluate oil quality. The GC-MS analysis of the essential oil samples was carried out using gas chromatography-mass spectrometry instrument stands at the Department of Medicinal and Aromatic Plants Research, National Research Center, Egypt. Most of the compounds were

identified using mass spectra (authentic chemicals, Wiley spectral library collection and NSIT library).

Statistical Analyses and Data Interpretation: In present study many statistical analyses were used to achieve the objectives of the study. Analysis of variance (ANOVA) for studied traits, mean comparisons, polynomial regression analysis, correlation and stepwise multiple linear regression analysis were conducted by using the statistical software packages; (SPSS) version 17.0 software [25], MSTAT-C computer programmed [26].

Analysis of Variance and Mean Comparisons: Individual analysis of variance was performed for all traits of each year according to the procedure described by Gomez and Gomez [27] for the split plot design. Error mean squares were tested for variance heterogeneity using Bartlett's [28] method and combined analysis of variance over each cut was done, for all studied traits according to Steel *et al.* [29]. Duncan's multiple range test (DNMRT) at 5% level of probability was used to find significant differences among means according to Duncan [30].

Polynomial and Nonlinear Models: Polynomial regression analysis was used to study the relationships among different nickel and proline levels. Linear and quadratic orthogonal polynomials were tested using appropriate regression models to examine the response of basil plants to increasing nickel and proline levels. The least squares procedure was applied to develop linear and quadratic models according to Snedecor and Cochran [31]. The fundamental goal is to find the best model that best fits the data. If the trend is statistically significant at $P = .05$, then the best model chosen from models including a linear and quadratic trend factor.

Correlation and Stepwise Multiple Linear Regression Analysis: Simple correlation coefficients were estimated by means between each of the dependent and independent variables according to Snedecor and Cochran [31]. Stepwise multiple linear regression analysis was performed according to Draper and Smith [32] for determination of the best model, which accounted for most of the variation, existed in dependent variable. Stepwise program computed a sequence of multiple linear regression in a stepwise manner. One variable was added to the regression equation at each step. The added variable was the one which induced the highest values of error sum of squares [33].

RESULTS AND DISCUSSION

Combined Analysis of Variance: Results of the combined analysis of variance for all studied traits, are presented in Tables 1 and 2. Mean squares (MS) for different sources of variation showed that years (Y) effect was non-significant ($P \leq 0.05$) for all the studied traits in both cuts except, plant diameter (1st and 2nd cuts), plant height (2nd cut). By looking at the different factors tested, it is clear that there were highly significant ($P \leq 0.01$) influences of proline (P) and nickel rates (Ni) on all studied traits in both cuts. Data presented in Tables 1 and 2 revealed that the interaction effect between proline and nickel rates exhibited highly significant ($P \leq 0.01$) variations for all studied indicating that the two factors are dependent on each other.

Effect of Proline and Nickel Rates on the Studied Traits Across Two Years

Essential Oil Yield Ton/Fed

Effect of Proline: The mean performance of studied traits is presented in Tables 3 and 4. There was an increase in oil yield L/fed with increasing proline rates from 0 to 100 ppm in both cuts, but application of 100 ppm caused a decline in oil yield L/fed. In the present study oil yield L/fed ranged from 11.640 to 17.687 and from 12.013 to 18.092 L/fed for the 1st and 2nd cut, respectively. The highest oil yield was obtained by applying 50 ppm and the lowest oil yield was produced from untreated plants in both cuts. This result clearly pointed out the importance of proline for higher oil yield production in basil. The maximum oil yield at 50 ppm was attributed to the improvement in the all studied traits (Tables 3 and 4). However, there were insignificant differences between proline rates of 50 ppm and 100 ppm for all yield attributes in both cuts such as fresh herb yield, dry herb yield, fresh leaves yield, dry leaves yield, fresh and dry flowering tops yields.

The increments in magnitude of examined traits created by 50 and 100 ppm of proline compared with control plants might be due to increase in proline accumulation, which not just protects enzymes, 3D structures of proteins and organelle membranes, yet it likewise supplies energy for growth and survival thereby helping the plant to endure stress [34, 35]. However, some researchers reported that high doses of proline may be harmful to plants, including inhibitory effects on growth or deleterious effects on cellular metabolisms [36, 37].

These findings of the present study are similar to some studies in *Allenrolfea occidentalis* [38].

The statistical model which defines this relation between proline rates and oil yield of basil was obtained using the quadratic function in both cuts across two seasons (Tables 3 and 4). According to the regression analysis results, proline rates used to obtain the highest total production is shown in Fig. 1 and 2.

The response of oil yield to proline application was quadratic (Fig. 1) showed the importance of this element in the production system. High R^2 (0.99) indicates a close relationship between oil yield and proline rates. The measured and predicted values versus proline were shown in Fig. 1. The obtained model is formulated as:

$$y = -0.0019x^2 + 0.2139x + 11.64, R^2=0.99 \text{ (1}^{\text{st}} \text{ cut)} \text{ and } y = -0.0017x^2 + 0.2068x + 12.013 R^2 = 0.99 \text{ (2}^{\text{nd}} \text{ cut)}$$
 where Y is oil yield in L/fed and x is applied proline in ppm.

Effect of Nickel: Nickel rates had significant effect on the oil yield of basil in both cuts (Tables 3 and 4). Average oil yield ranged from 11.812 to 14.715 L/fed and from 12.853 to 15.299 L/fed in the 1st and 2nd cut, respectively. In general, the oil yield increments with 50 ppm over those of 100 ppm in 1st and 2nd cuts were 14.36% and 13.6%, respectively. These increments in oil yield with the 50 ppm were mainly attributed to the higher values of all yield attributes studied through both cuts. Similar finding about Ni effect were reported by Seregin and Kozhevnikova [18] who reported that the small quantities of Ni (0.01 to 5 μ g/g dry wt) is essential for many plants to complete their life cycle but higher concentrations of this metal are toxic and may severely interfere with many physiological and biochemical processes of plants. The earliest report of a growth response to Ni application indicated that Ni deficiency has a wide range of effects on plant growth, plant senescence, N metabolism and Fe uptake [18, 39]. As well as Atta-Aly [19] showed that low levels of Ni fertilization particularly 50 mg/kg soil increased parsley leaf yield and quality without affecting leaf chlorophyll and Fe contents.

The relationship between oil yield and nickel rates represented a quadratic and followed the equations: $y = -0.0016x^2 + 0.188x + 11.812 R^2 = 0.98$ for the 1st cut (Fig 3) and $y = -0.0014x^2 + 0.1653x + 12.853, R^2 = 0.98$ in the 2nd cut (Fig. 4). The quadratic response of oil yield to nickel rates showed the importance of this factor in the production of oil.

Table 1: Combined analysis of variance results and significance for different traits of basil evaluated across two years under different nickel and proline rates for the first cut

MS										
S.O.V	df	Plant height	Plant diameter	Fresh herb yield	Dry herb yield	Fresh leaves yield	Dry leaves yield	Fresh flowering tops yield	Dry flowering tops yield	Oil yield
Years (Y)	1	1.707ns	1.402*	0.373ns	0.022ns	0.045ns	0.001ns	0.041ns	0.005ns	3.385*
Replicates(Y)	4	0.282	1.040	0.462	0.039	0.059	0.002	0.080	0.010	2.239
Proline (P)	2	56.885**	36.426**	7.405**	0.553**	0.929**	0.028**	1.033**	0.123**	164.868**
Y x P	2	0.637ns	0.074ns	0.009ns	0.000ns	0.001ns	0.000ns	0.004ns	0.000ns	1.150ns
Error	8	0.662	0.235	0.106	0.009	0.014	0.001	0.024	0.003	0.455
Nickel (N)	2	123.195**	325.326**	20.877**	1.633**	2.644**	0.082**	2.929**	0.337**	132.655**
Y x N	2	0.262ns	0.227ns	0.199ns	0.020ns	0.025ns	0.001ns	0.023ns	0.002ns	0.020ns
P x N	4	24.844**	36.990**	2.513**	0.201**	0.316**	0.010**	0.333**	0.039**	23.651**
Y x P x N	4	0.281ns	0.181ns	0.194ns	0.016ns	0.024ns	0.001ns	0.023ns	0.002ns	0.712ns
Error	24	0.344	0.596	0.126	0.010	0.016	0.001	0.018	0.002	0.290
C.V.%		0.96%	1.93%	2.98%	3.05%	2.97%	3.15%	2.91%	2.89%	3.69%

ns, * and ** show insignificance and significance at 5 and 1% probability level, respectively.

Table 2: Combined analysis of variance results and significance for different traits of basil evaluated across two years under different nickel and proline rates for the second cut.

MS										
S.O.V	df	Plant height	Plant diameter	Fresh herb yield	Dry herb yield	Fresh leaves yield	Dry leaves yield	Fresh flowering tops yield	Dry flowering tops yield	Oil yield
Years (Y)	1	7.114**	4.860**	0.028Ns	0.002ns	0.000ns	0.000ns	0.000ns	0.000ns	0.339ns
Replicates(Y)	4	0.367	0.705	0.431	0.034	0.037	0.001	0.043	0.004	1.386
Proline (P)	2	72.036**	39.031**	14.868**	1.155**	1.966**	0.061**	2.255**	0.238**	168.356**
Y x P	2	2.503*	0.272ns	1.616**	0.126*	0.159*	0.005ns	0.190*	0.023*	3.659**
Error	8	0.538	0.171	0.216	0.017	0.035	0.001	0.040	0.004	0.121
Nickel (N)	2	119.191**	328.285**	16.650**	1.303**	1.974**	0.060**	2.268**	0.267**	101.327**
Y x N	2	1.100*	0.401ns	0.398Ns	0.030ns	0.075ns	0.002ns	0.085ns	0.013ns	1.386ns
P x N	4	24.988**	34.458**	4.822**	0.379**	0.594**	0.019**	0.682**	0.082**	26.619**
Y x P x N	4	0.412ns	0.743*	0.920**	0.072**	0.078ns	0.002ns	0.090ns	0.010ns	1.004ns
Error	24	0.324	0.271	0.188	0.015	0.033	0.001	0.038	0.005	0.873
C.V.%		0.92%	1.30%	3.53%	3.55%	4.18%	3.93%	4.16%	4.35%	6.13%

ns, * and ** show insignificance and significance at 5 and 1% probability level, respectively.

Table 3: Means comparison for the main effects of proline and nickel rates for studied traits of basil based for the 1st cut across two years on Duncan's multiple range test (DMRT) at 5% probability level

Treatments	Plant height cm	Plant diameter cm	Fresh herb yield ton/fed. ⁺¹	Dry herb yield ton/fed.	Fresh leaves yield ton/fed.	Dry leaves yield ton/fed.	Fresh flowering tops yield ton/fed.	Dry flowering tops yield ton/fed.	Oil yield L/fed.
Proline									
0 ppm	60.710b	39.090b	11.197b	3.142b	3.975b	0.701b	4.266b	1.449b	11.640c
50 ppm	63.360a	41.570a	12.374a	3.464a	4.392A	0.774a	4.700a	1.598a	17.687a
100 ppm	59.980c	39.110b	12.227a	3.423a	4.339A	0.762a	4.658a	1.584a	14.437b
Regression model									
Linear	*	Ns	**	**	**	**	**	**	**
Quadratic	**	**	**	**	**	**	**	**	**
Nickel									
0 ppm	58.480c	35.030c	11.012c	3.082c	3.908C	0.688c	4.194c	1.426c	11.812c
50 ppm	63.590a	42.680a	13.117a	3.673a	4.657A	0.820a	4.984a	1.694a	17.237a
100 ppm	61.980b	42.070b	11.669b	3.274b	4.141b	0.729b	4.445b	1.512b	14.715b
Regression model									
Linear	**	**	**	**	**	**	**	**	**
Quadratic	**	**	**	**	**	**	**	**	**

Means followed by similar letters in each column for each main nitrogen rate or plant distance are not significantly different at the 5 % probability level. ** Significant at 1% probability level and ns: not significant.

+1 feddan (fed.)=local area in Egypt equal 4200 m². 1 hectare = 2.4 feddan

Table 4: Means comparison for the main effects of proline and nickel rates for studied traits of basil based for the 2nd cut across two years on Duncan's multiple range test (DMRT) at 5% probability level

Treatments	Plant height cm	Plant diameter cm	Fresh herb yield ton/fed. ⁺¹	Dry herb yield ton/fed.	Fresh leaves yield ton/fed.	Dry leaves yield ton/fed.	Fresh flowering tops yield ton/fed.	Dry flowering tops yield ton/fed.	Oil yield L/fed.
Proline									
0 ppm	60.510b	39.240b	11.239b	3.148b	3.989b	0.702b	4.283b	1.465b	12.013c
50 ppm	63.880a	41.860a	12.725a	3.563a	4.548a	0.801a	4.881a	1.660a	18.092a
100 ppm	60.320b	39.390b	12.888a	3.608a	4.574a	0.806a	4.910a	1.668a	15.644b
Regression model									
Linear	Ns	**	**	**	**	**	**	**	**
Quadratic	**	**	**	**	**	**	**	**	**
Nickel									
0 ppm	58.720c	35.240c	11.626c	3.255c	4.157c	0.732b	4.462b	1.517b	12.853c
50 ppm	63.730a	42.860a	13.388a	3.748a	4.752a	0.836a	5.100a	1.738a	17.597a
100 ppm	62.260b	42.390b	11.839b	3.316b	4.203b	0.740b	4.511b	1.538b	15.299b
Regression model									
Linear	**	**	**	**	ns	ns	ns	ns	**
Quadratic	**	**	**	**	**	**	**	**	**

Means followed by similar letters in each column for each main nitrogen rate or plant distance are not significantly different at the 5 % probability level. ** Significant at 1% probability level and ns: not significant +1 feddan (fed.)=local area in Egypt equal 4200 m². 1 hectare = 2.4 feddan

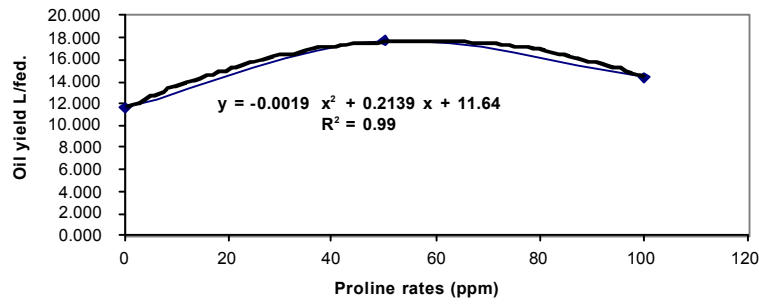


Fig. 1: Measured and predicted oil yield (L/fed) values versus proline rates in the 1st cut

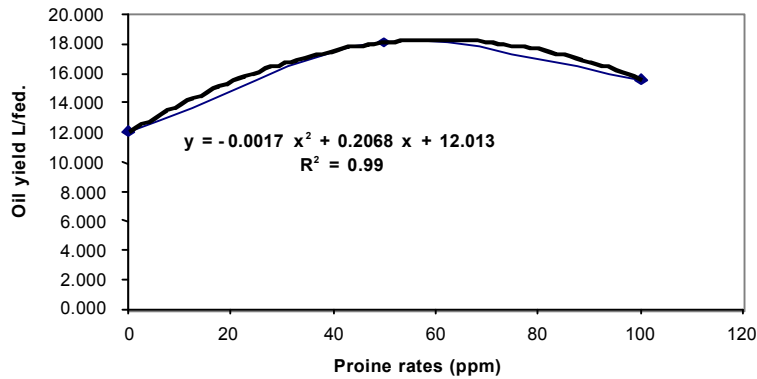


Fig. 2: Measured and predicted oil yield (L/fed) values versus proline rates in the 2nd cut

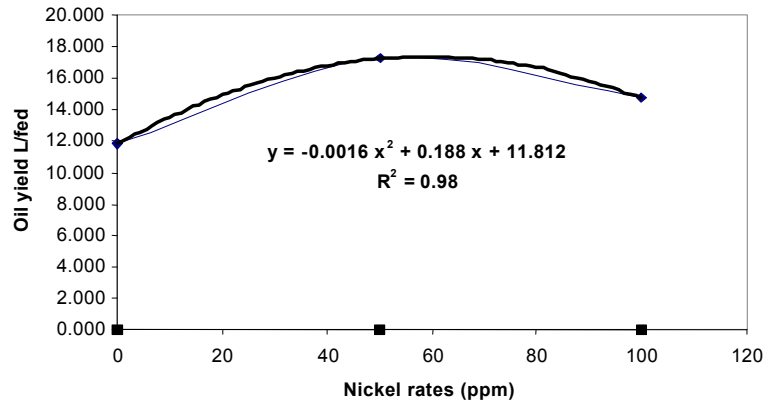


Fig. 3: Measured and predicted oil yield (L/fed) values versus nickel rates in the 1st cut

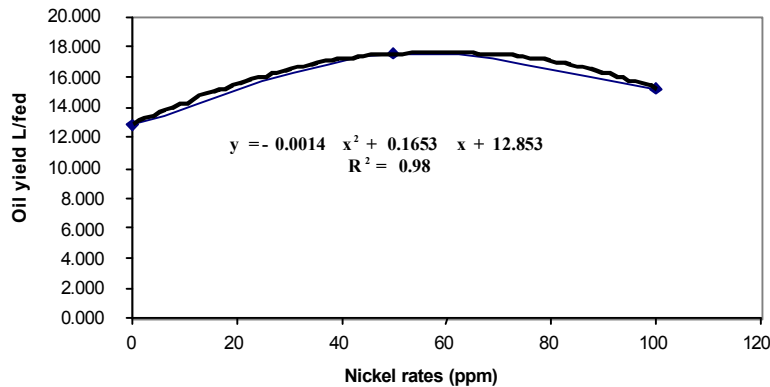


Fig. 4: Measured and predicted oil yield (L/fed) values versus nickel rates in the 2nd cut

Fresh and Dry Yield and its Related Traits:

Effect of Proline: Fresh and dry yield is the major economical product of basil. Significant variations on fresh and dry yield were observed among proline rates in both cuts across two seasons. The results showed that maximum fresh and dry herb yield (12.374 and 3.464 ton/fed) in 1st cut and (12.725 and 3.563 ton/fed) in 2nd cut were obtained by applying 50 ppm, while the minimum measurements (11.197 and 3.142 ton/fed) in 1st cut and (11.239 and 3.148 ton/fed) in 2nd cut were recorded in untreated plants (Tables 3 and 4). However, there were insignificant differences between the effect of 50 and 100 ppm of proline on fresh and dry yields of herb in both cuts. Also, the results in Tables 3 and 4 showed the same trend for fresh leaves yield ton/fed, dry leaves yield ton/fed, fresh and dry flowering tops yields ton/fed in both cuts. These results agreed with the findings of Chandrashekar and Sandhyarani [34], Hoque *et al.* [40], Ashraf and Foolad [5], Deivanai [37] and Soha and El-Noemani [35].

The statistical model which defines this relation between using proline rates and all yield attributes of basil was obtained using the quadratic function in both cuts across two seasons. Meanwhile, to the regression analysis results, proline rates used to obtain the highest total production is shown in Figures 5 and 6.

Effect of Nickel: In the present study, fresh and dry yield with various nickel rates were measured. The results indicated that significant effect of nickel on fresh and dry herb yields. Maximum fresh and dry herb yields (13.117 and 3.673 ton/fed) in 1st cut and (13.388 and 3.748 ton/fed) in 2nd cut were obtained at 50 ppm, against the (11.012 and 3.082 ton/fed) in 1st cut and (11.626 and 3.255 ton/fed) in 2nd cut.

The relationship between all yield traits and nickel rates represented as quadratic function (Figures 7 and 8).

Growth Traits: Effect of proline: Mean performance of plant height and plant diameter are displayed in Tables 3 and 4. The results showed that maximum plant height and plant diameter in both cuts were acquired by applying 50 ppm proline while the minimum measurements were recorded by applying 100 ppm proline. In any case, no significant differences were seen in plant height or plant diameter in both cuts between the untreated plants and the plants treated with 100 ppm of proline except plant height in 1st cut. These outcomes concur with the findings

of Chandrashekar and Sandhyarani [34], Hoque *et al.* [40], Ashraf and Foolad [5], Deivanai *et al.* [37] and Soha and El-Noemani [35].

The statistical model which defines this relation between proline rates and plant height or plant diameter of basil was obtained using the quadratic function in both cuts as shown in Figures 9 and 10.

Effect of Nickel: The results indicated that significant effect of nickel on plant height and plant diameter. Maximum plant height and plant diameter (63.590 and 42.680 cm) in 1st cut and (63.730 and 42.860 cm) in 2nd cut were obtained at 50 ppm, against the minimum measurements (58.480 and 35.030 cm) in 1st cut and (58.720 and 35.240 cm) in 2nd cut.

The relationship between all yield traits and nickel rates represented as quadratic function (Figures 11 and 12).

Interactive Effects of Different Proline and Nickel Rates:

Interactive effects of different proline and nickel rates on all studied traits were highly significant ($p=0.01$) in both cuts across two years. According to the results in Tables 5 and 6, oil yield values ranged from 8.578 to 22.538 L/fed in the 1st cut and from 8.487 to 22.450 L/fed in the 2nd cut across 2 years. Maximum oil yield was recorded when proline was applied at 50 ppm and nickel at 50 ppm. The minimum oil yield was recorded when the proline was applied at 0 ppm and nickel of 0 ppm. All remain studied traits (plant height, plant diameter, fresh herb yield, dry herb yield, fresh leaves yield, dry leaves yield, fresh flowering tops yield and dry flowering tops yield) behaved the same trend as oil yield since the maximum values were recorded with 50 ppm proline and 50 ppm nickel while the minimum values were achieved from untreated plants. Maximum oil yield was recorded when proline was applied at 50 ppm and nickel of 50 ppm. Therefore, it is suggested that among different proline rates (0, 50 and 100 ppm) and different nickel rates (0, 50 and 100 ppm), proline of 50 ppm with 50 ppm of nickel per feddan are optimum for basil plant under agro-ecological conditions of Giza, Egypt.

Concerning the effect of Ni it was reported by Chand [41] that Ni and Pb applied at 25:25 produced 40 % higher fresh herbage and root of *Mentha arvensis* L. while 50:50 ppm exhibited negative effect on the herb and yield. Also, Teixeira *et al.* [39] proved that Ni deficiency affects plant growth, plant senescence, nitrogen metabolism and iron

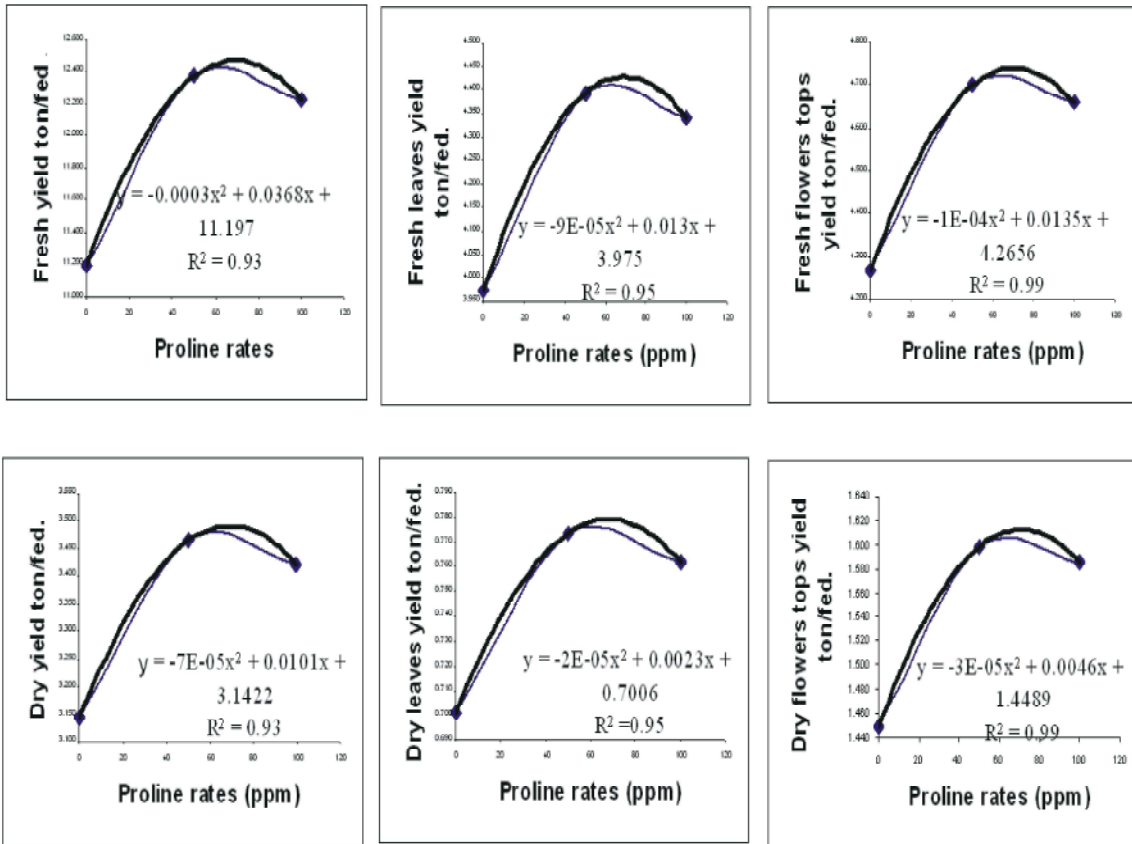


Fig. 5: Measured and predicted yield traits (ton/fed) values versus proline rates in the 1st cut

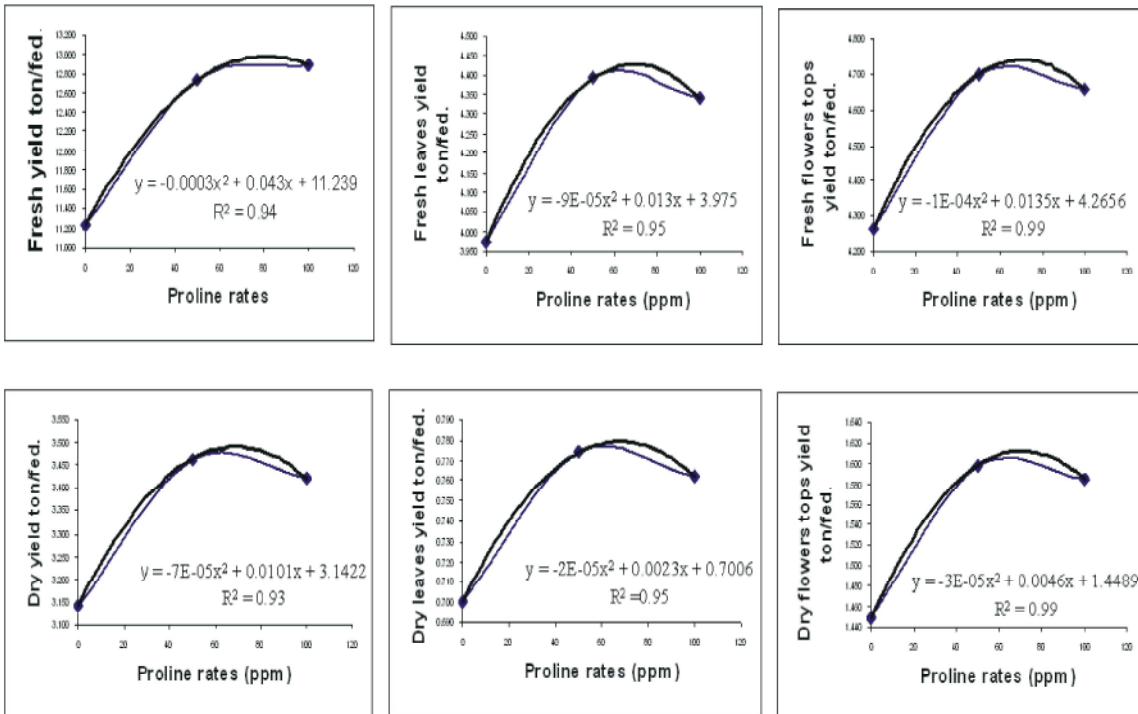


Fig. 6: Measured and predicted yield traits (ton/fed) values versus proline rates in the 2nd cut

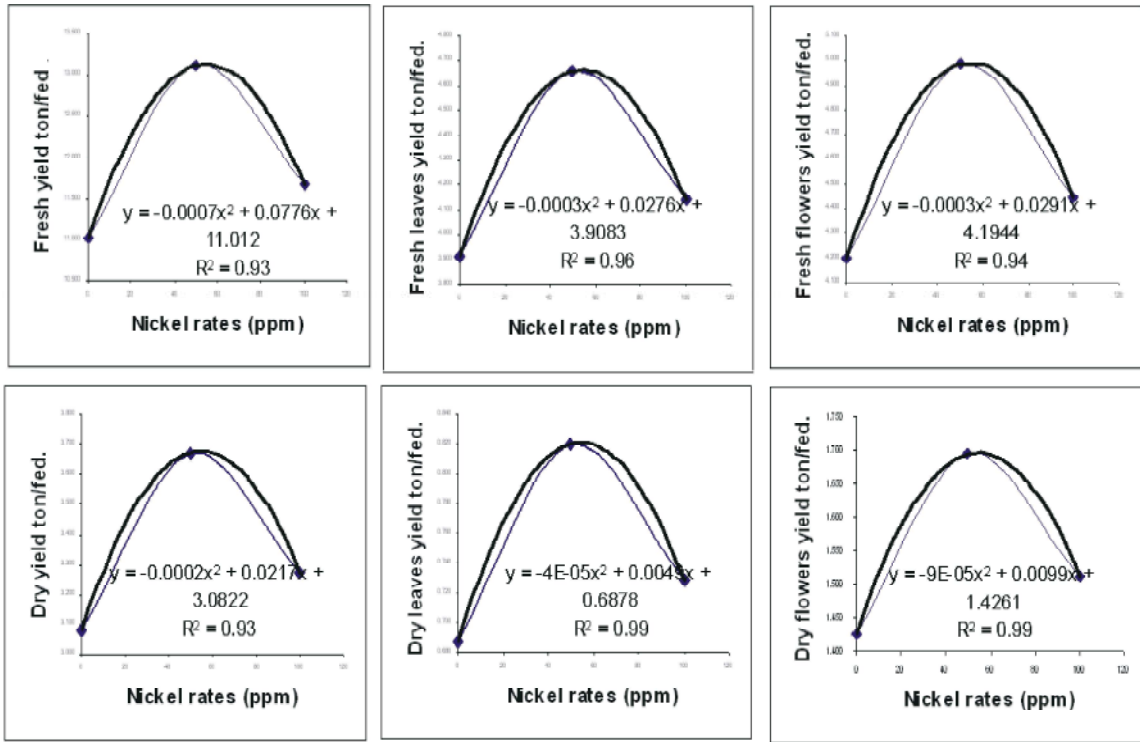


Fig. 7: Measured and predicted yield traits (ton/fed) values versus nickel rates in the 1st cut

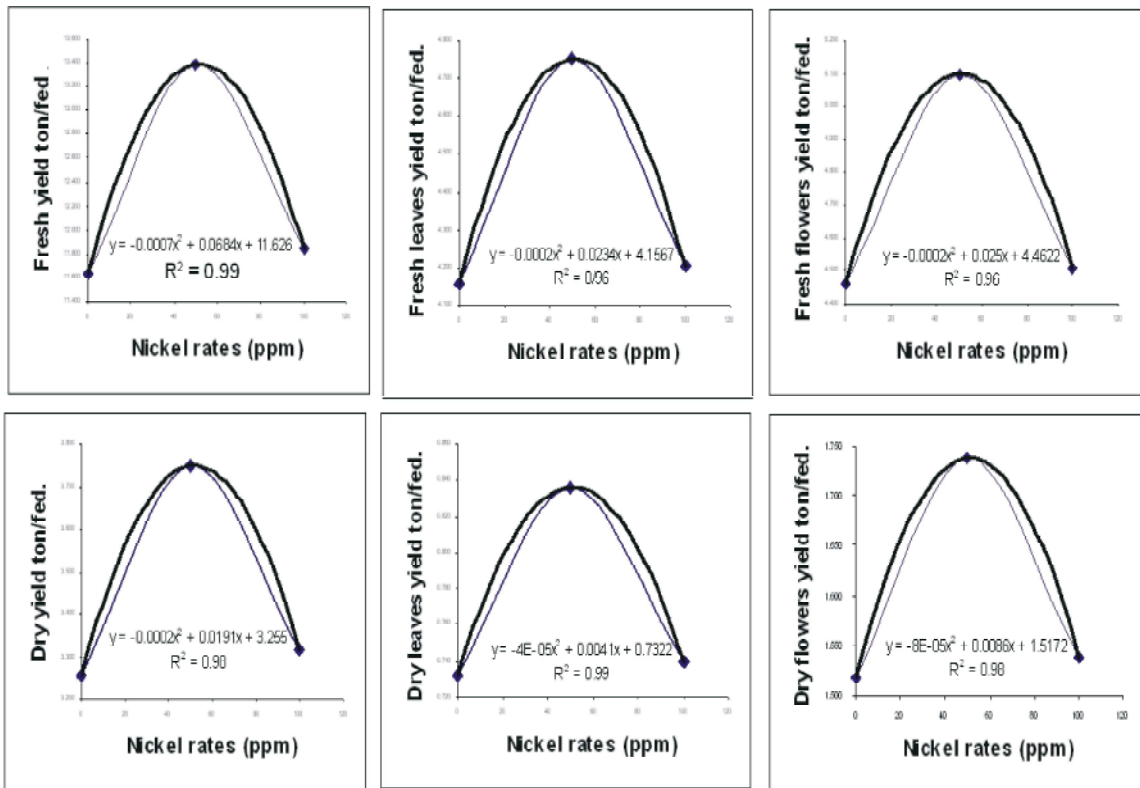


Fig. 8: Measured and predicted yield traits (ton/fed) values versus nickel rates in the 2nd cut

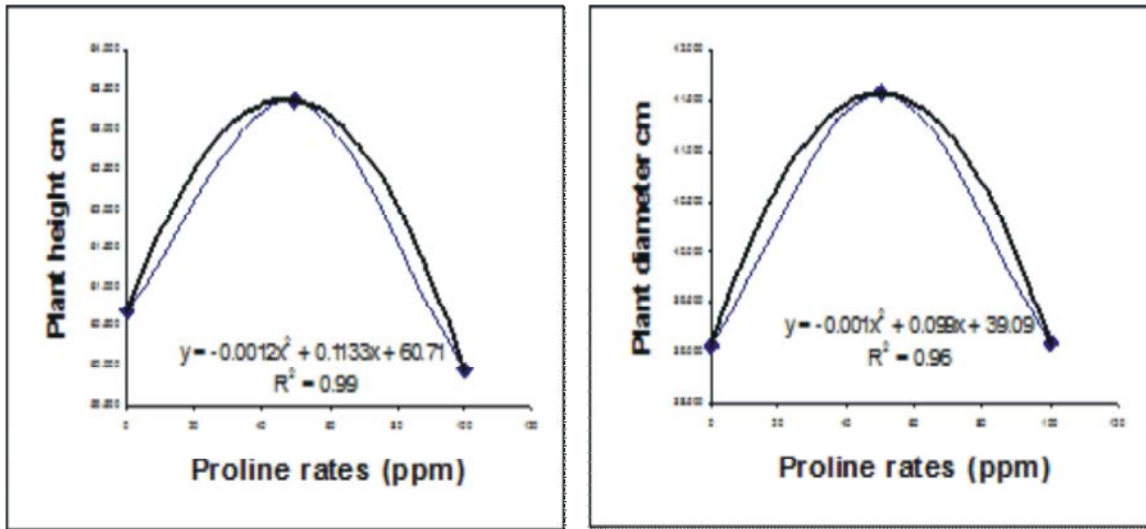


Fig. 9: Measured and predicted plant height and plant diameter (cm) values versus proline rates in the 1st cut

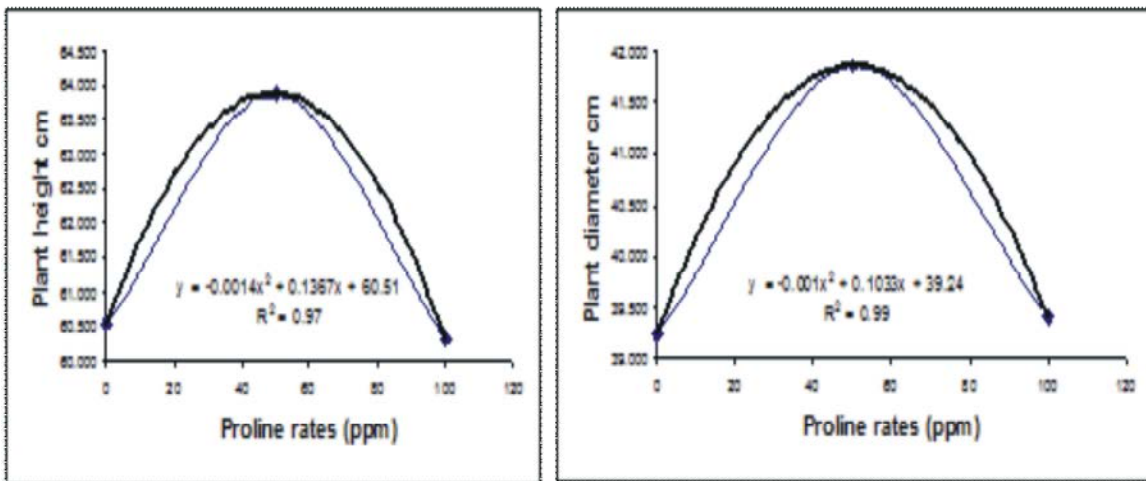


Fig. 10: Measured and predicted plant height and plant diameter (cm) values versus proline rates in the 2nd cut

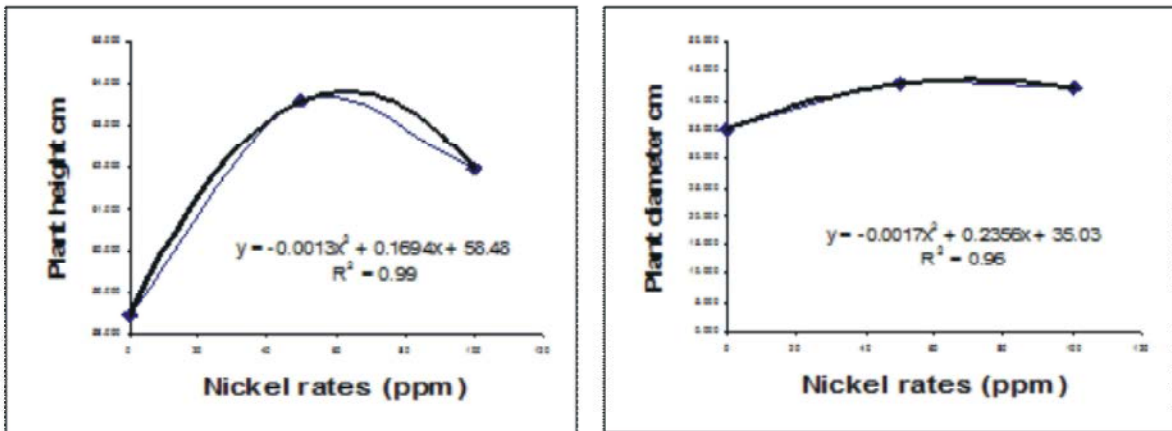


Fig. 11: Measured and predicted plant height and plant diameter (cm) values versus nickel rates in the 1st cut

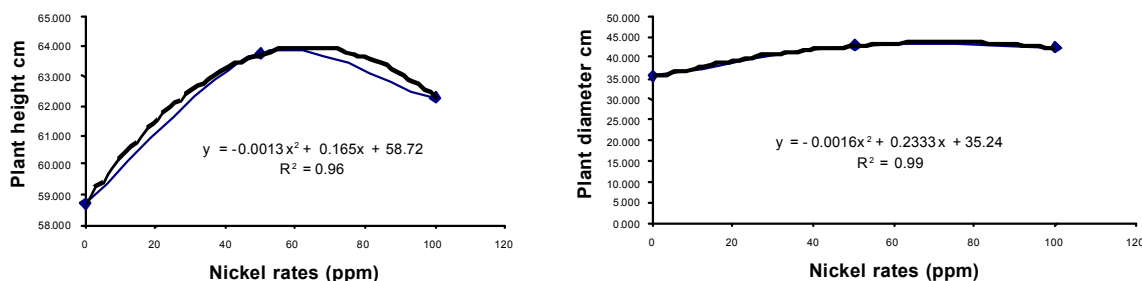


Fig. 12: Measured and predicted plant height and plant diameter (cm) values versus nickel rates in the 2nd cut

Table 5: Effect of proline and nickel rates on on studied traits of basil in the 1st cut across two years

Treatments	Plant height cm	Plant diameter cm	Fresh yield ton/fed.	Dry yield ton/fed.	Fresh leaves yield ton/fed.	Dry leaves yield ton/fed.	Fresh flowering tops yield ton/fed.	Dry flowering tops yield ton/fed.	Oil yield L/fed.
Proline Ni									
0	56.820g	32.620h	10.018f	2.805f	3.558e	0.625f	3.817f	1.297f	8.578f
50	61.550c	40.580e	12.350c	3.458c	4.385bc	0.775bc	4.707c	1.597c	12.810e
100	63.770b	44.080b	11.222e	3.163e	3.982d	0.702e	4.273e	1.453e	13.532cd
50	60.830d	36.130g	11.253e	3.148e	3.993d	0.705e	4.287e	1.457e	13.758c
100	65.500a	46.130a	14.268a	3.995a	5.067a	0.892a	5.395a	1.835a	22.538a
100	63.750b	42.430c	11.600d	3.250d	4.117cd	0.725de	4.418d	1.503d	16.765b
100	57.780f	36.330g	11.763d	3.293d	4.173bcd	0.733d	4.480d	1.525d	13.100be
100	63.730b	41.320d	12.732b	3.565b	4.520b	0.793b	4.850b	1.650b	16.363b
100	58.430e	39.680f	12.185c	3.410c	4.325bcd	0.760c	4.643c	1.578c	13.848c

Any two means not sharing a letter in common differ significantly at 5% probability level- using Duncan's multiple range test

Table 6: Effect of proline and nickel rates on on studied traits of basil in the 2nd cut across two years

Treatments	Plant height cm	Plant diameter cm	Fresh yield ton/fed.	Dry yield ton/fed.	Fresh leaves yield ton/fed.	Dry leaves yield ton/fed.	Fresh flowering tops yield ton/fed.	Dry flowering tops yield ton/fed.	Oil yield L/fed.
Proline Ni									
0	57.100g	33.000h	9.870f	2.765f	3.502f	0.617f	3.760f	1.280f	8.487f
50	60.930d	40.570e	12.475c	3.493c	4.428c	0.778c	4.755c	1.627c	13.277e
100	63.500c	44.150b	11.372e	3.187e	4.038e	0.712e	4.333e	1.488e	14.277d
50	61.020d	36.180g	11.848d	3.317d	4.298cd	0.757cd	4.613cd	1.568cd	15.278c
100	66.120a	46.400a	14.535a	4.070a	5.160a	0.908a	5.535a	1.883a	22.450a
100	64.500b	43.000c	11.792d	3.302d	4.187de	0.737de	4.493de	1.528de	16.547b
100	58.050f	36.530g	13.158b	3.683b	4.670b	0.823b	5.013b	1.703b	14.793cd
100	64.130b	41.600d	13.153b	3.682b	4.668b	0.822b	5.010b	1.703b	17.065b
100	58.780e	40.030f	12.353c	3.458c	4.385c	0.772c	4.707c	1.598c	15.073cd

Any two means not sharing a letter in common differ significantly at 5% probability level- using Duncan's multiple rang

uptake and it may play a role in disease resistance, but also excessive Ni inhibits and development of plants. In addition, Atta-Aly [19] on parsley and Helmy [20] on coriander reported that low levels of Ni fertilization increased leaf yield and quality.

Effect of Proline and Nickel Rates on the Chemical Constituents of Basil Essential Oil: Essential oil components in basil herb were listed in Table 7 where 17 compounds were recognized. In control plants, linalool (36.69 %), 1, 8 cineole (17.39 %), α -bergamotene (14.24 %) were the primary components. tau cadinol, γ -cadinol, eugenol, β fenchyl alcohol were available in little amounts and other compounds were beneath 2%.

Results demonstrated that basil essential oil components were influenced by proline and nickel applications. Among all treatments, 50 ppm proline brought about highest contents of linalool and the least contents of 1, 8 cineole. The most noteworthy 1, 8-cineole were gotten without proline application.

Likewise, treating plants with 50 ppm nickel prompted an increase in linalool content compared to the control then 100 ppm nickel declined it. The rest of components were not different from one another.

Then again, use of 50 ppm Ni + 50 ppm proline expanded the relative percent of linalool which achieved 42.88 % while 1, 8 cineole sharply declined by this treatment.

Table 7: Effect of proline and nickel rates on the chemical constituents of basil essential oil

Compound	N0P0	N0P1	N0P2	N1P0	N1P1	N1P2	N2P0	N2P1	N2P2
α-pinene	0.57	t	0.41	0.73	t	t	1	T	0.54
β-pinene	1.23	0.73	0.89	1.37	t	t	1.79	0.32	1.2
1, 8 Cineole	17.39	13.93	14.61	16.53	8.2	8.64	18.74	7.78	15.69
n-octyl acetate	1.10	1.16	1.23	1.07	1.4	1.01	1.4	1.27	1.04
Linalool	36.69	38.57	37.14	39.15	42.88	39.45	35.91	41.69	37.67
α-Bergamotene	14.24	15.27	14.68	14.23	14.99	15.36	14.06	15.39	14.15
β- elemene	0.63	0.77	0.74	0.93	0.65	0.9	0.64	0.64	0.89
α-caryophyllene	1.17	1.45	1.33	1.27	1.46	1.65	1.17	1.37	1.41
β farnesene	0.63	0.78	0.67	0.58	0.71	0.71	0.66	0.72	0.74
Germacrene d	1.05	1.77	1.34	0.99	1.82	1.75	1.07	1.51	1.83
β fenchyl alcohol	2.51	1.74	2.12	1.72	2.37	2.2	1.85	2.5	1.6
γ-cadinol	3.28	3.7	3.69	3.38	3.48	4.18	3.57	3.74	3.52
Vediflorol	1.44	1.6	2.07	1.13	1.76	2.14	1.04	1.56	1.48
Bergamotol z	0.37	0.36	0.42	0.37	0.4	0.46	0.34	0.41	0.36
Cubenol	1.17	1.2	1.35	1.09	1.3	1.51	1.06	1.42	1.19
Spathulenol	0.81	0.75	0.92	0.8	0.88	1.12	0.71	0.93	0.82
Tau cadinol	7.14	7.46	8.31	6.61	7.97	9.52	6.52	8.82	7.41
Eugenol	2.50	2.49	2.23	2.14	2.66	2.64	2.46	3.53	1.7

*t traces

Correlation and Stepwise Regression Analysis:

Correlation analysis: Simple correlation coefficient is one of the important indicators to study the nature of the correlations between traits for use in plant improvement following appropriate method of selection. Tables 8 and 9 showed the matrix of correlation coefficient describing the relationships between studied traits for cut 1 and 2, respectively.

Results showed plant height, plant diameter, fresh yield, dry yield, fresh leaves yield, dry leaves yield, fresh flowering tops yield and dry flowering tops yield have positive and highly significant relationships oil yield in both cuts the where, Pearson’s coefficients were (0.759**, 0.741**), (0.776**, 0.732**), (0.867**, 0.853**) (0.869**, 0.853**), (0.867**, 0.852**), (0.864**, 0.853**), (0.859**, 0.850**) and (0.862**, 0.848**) in the 1st and 2nd cuts, respectively (Tables 7 and 8). These results imply that all traits contribute to increase in oil yield. These relationships need to be considered by the basil breeder, when making selections for the isolation of superior genotypes with desirable traits. However, the matrix of correlation showed a positive and highly significant among all possible combinations of studied traits. Also, there were full correlation coefficients (1.00) for many relations in Tables 8 and 9, indicating that these traits are identical and we can use any one of them to identify the other.

Our results confirm the findings of Safavi *et al.* [21] on safflower reported that seed yield was positively correlated with number of heads plant⁻¹, seed yield

plant⁻¹ and 1000-seed weight except days to 50% maturity. Also similar results were confirmed with Abd El-Mohsen and Gamalat [23] on safflower.

Modeling of Total Essential Oil Yield Based on Stepwise Multiple Linear Regressions:

The regression modeling was conducted based on stepwise regression. In order to indentify the traits (explanatory variables) those are important for oil yield. The results of stepwise regression analysis in basil under proline and nickel rates was calculated by considering the oil yield as the dependent variable and other traits as the independent variables.

The regression analysis of oil yield (l/fed) as dependant variable (Tables 10 and 11) according to stepwise method demonstrated that traits such as plant height, fresh leaves yield, fresh flowering tops yield and dry flowering tops yield in the 1st cut entered to regression model and totally justified 87.51 % of the variation that existed in oil yield. Meanwhile traits such as plant height, fresh leaves yield and fresh flowering tops yield in the 2nd cut entered to regression model and totally justified 87.30 % of the variation that existed in oil yield. The unexplained variation (12.49 and 12.79 % of the total for 1st and 2nd cut, respectively) may be due to variation in other components. These results corroborate with the path analysis and stepwise regression reported earlier for yield parameters of safflower by Pandia *et al.* [42] and Omidi [43]

At last, the following regression model was obtained for indicating the relationship between the oil yield and other traits as independent variables:

Table 8: Matrix of correlation coefficients between studied traits of basil under different proline and nickel rates in the 1st cut (Data are combined across treatments and seasons)

Traits	X ₁	X ₂	X ₃	X ₄	X ₅	X ₆	X ₇	X ₈	X ₉
Plant height cm (x ₁)	1	0.863**	0.569**	0.576**	0.568**	0.572**	0.563**	0.564**	0.759**
Plant diameter cm (x ₂)		1	0.675**	0.685**	0.674**	0.675**	0.672**	0.673**	0.776**
Fresh yield ton/fed (x ₃)			1	0.998**	1.000**	0.998**	0.997**	0.997**	0.867**
Dry yield ton/fed (x ₄)				1	0.998**	0.997**	0.996**	0.996**	0.869**
Fresh leaves yield ton/fed (x ₅)					1	0.998**	0.997**	0.997**	0.867**
Dry leaves yield ton/fed (x ₆)						1	0.995**	0.995**	0.864**
Fresh flowering tops yield ton/fed (x ₇)							1	1.000**	0.859**
Dry flowering tops yield ton/fed (x ₈)								1	0.862**
Oil yield L/fed (x ₉)									1

** , significant at 1% level of probability, respectively.

Table 9: Matrix of correlation coefficients between studied traits of basil under different proline and nickel rates in the 2nd cut (Data are combined across treatments and seasons)

Traits	X ₁	X ₂	X ₃	X ₄	X ₅	X ₆	X ₇	X ₈	X ₉
Plant height cm (x ₁)	1	0.858**	0.470**	0.470**	0.467**	0.468**	0.465**	0.474**	0.741**
Plant diameter cm (x ₂)		1	0.532**	0.533**	0.510**	0.510**	0.508**	0.520**	0.732**
Fresh yield ton/fed (x ₃)			1	1.000**	0.982**	0.983**	0.982**	0.980**	0.853**
Dry yield ton/fed (x ₄)				1	0.982**	0.983**	0.982**	0.980**	0.853**
Fresh leaves yield ton/fed (x ₅)					1	0.999**	1.000**	0.996**	0.852**
Dry leaves yield ton/fed (x ₆)						1	0.999**	0.996**	0.853**
Fresh flowering tops yield ton/fed (x ₇)							1	0.996**	0.850**
Dry flowering tops yield ton/fed (x ₈)								1	0.848**
Oil yield L/fed (x ₉)									1

** , significant at 1% level of probability, respectively.

Table 10: Final model and contributions of different traits to oil yield determined by stepwise regression in the 1st cut across two years

Variable	Regression Coefficient	Probability value
Plant height (cm) x ₁	0.46	0.000
Fresh leaves yield (ton/fed) x ₂	10.25	0.007
Fresh flowering tops yield (ton/fed) x ₃	-62.78	0.004
Dry flowering tops yield (ton/fed) x ₄	171.77	0.006

$$Y = -37.28 + 0.46 x_1 + 10.25 x_2 - 62.78 x_3 + 171.77 x_4$$

R-squared = 87.51 % and R-squared (adjusted for d.f.) = 86.47 % . * and ** , significant at 5 and 1% levels of probability, respectively.

Table 11: Final model and contributions of different traits to oil yield determined by stepwise regression in the 2nd cut across two years

Variable	Regression Coefficient	Probability Value
Plant height (cm) x ₁	0.49	0.000
Fresh leaves yield (ton/fed) x ₂	73.81	0.002
Fresh flowering tops yield (ton/fed) x ₃	-64.29	0.005

$$Y = -35.86 + 0.49 x_1 + 73.81 x_2 - 64.29 x_3$$

R-squared = 87.30 % and R-squared (adjusted for d.f.) = 86.52 % . * and ** , significant at 5 and 1% levels of probability, respectively.

$Y = -37.28 + 0.46 x_1 + 10.25 x_2 - 62.78 x_3 + 171.77 x_4$ (1st cut),
 $Y = -35.86 + 0.49 x_1 + 73.81 x_2 - 64.29 x_3$ (2nd cut). In these equations Y is the oil yield; x₁, x₂, x₃ and x₄ are plant height, fresh leaves yield, fresh flowering tops yield and dry flowering tops yield, respectively (Tables 10 and 11).

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