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Meta-Analysis of the Effect of Drought Stress on Quantitative and Qualitative Yield of *Nigella Sativa*

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ABSTRACT
This study was conducted in order to investigate the effect of drought stress on the quantitative and qualitative yield of black seed with meta-analysis method. The research sample included a collection of domestic articles and theses between 1991 and 2021, and a total of 18 articles and theses were found to be appropriate. Of these, 18 articles had a control treatment or no drought stress. 12 articles were in the range of 90-81 and 40-60% and 14 articles were 80-61% of farm capacity. Mean and mean square of 6 traits including seed yield, biological yield, seed protein, seed oil, protein yield and oil yield were extracted. All statistical calculations and graphs were done with Excel software. The results showed that according to the standardized values, the effect of drought stress on grain yield and dry matter yield was significant. Regarding the percentage of oil and percentage of protein, the results showed that the average effect size was not significantly different from the control treatment and therefore drought stress did not have a significant effect on increasing or decreasing the percentage of oil and percentage of protein compared to the control treatment. The standardized values of the effect of drought stress on oil yield were significant compared to the control treatment and the control treatment with 40-60% of crop capacity. There was no significant difference between the control treatment and the 81-90% crop capacity treatment. Combining the data of the investigated experiments with the regression method showed that the reaction of grain yield to drought stress is consistent with the first-order model. With the increase of drought stress up to 40% of the crop capacity, the grain yield decreased linearly. The correlation coefficient between drought stress and black seed yield was 0.46. The correlation results also showed that the relationship between these two dependent and independent variables is significant. The results of this research showed that despite numerous studies on the effect of drought stress on the performance of black seed plant in the country, the results are very diverse and highly scattered. This has led to the fact that the effect of drought stress, especially on the percentage of oil and black seed, is still unclear. As a powerful statistical method, meta-analysis provided a suitable and accurate tool for combining the results of independent experiments and defined specific ranges for the effect of drought stress on the characteristics of black currents. Drought stress has harmful effects on the

quality of black seed and it is necessary to think of appropriate solutions to reduce the effect of drought stress on the quality of this valuable medicinal plant.

Keywords: Stress, Meta-Analysis, *Nigella Sativa*, Oil.

INTRODUCTION

Medicinal plants have been used for curing diseases for many centuries in different indigenous systems of medicine as well as folk medicines. Moreover, medicinal plants are also used in the preparation of herbal medicines as they are considered to be safe as compared to modern allopathic medicines. Many researchers are focusing on medicinal plants since only a few plant species have been thoroughly investigated for their medicinal properties, potential, mechanism of action, safety evaluation and toxicological studies (Rezaei *et al*., 2015; Fathi and Bahamin, 2018).

Nigella sativa (black caraway, also known as Siyahdaneh , black cumin, nigella or kalonji) is an annual flowering plant in the family Ranunculaceae, native to eastern Europe (Bulgaria and Romania) and Western Asia (Turkey, Iran and Iraq), but naturalized over a much wider area (Gharby *et al*., 2015), including parts of Europe, northern Africa and east to Myanmar. Oils are 32% to 40% of the total composition of *N.sativa* seeds *N.sativa* oil contains linoleic acid, oleic acid, palmitic acid, and trans-anethole, and other minor constituents, such as nigellicine, nigellidine, nigellimine, thymol, α-pinene, β-pinene and trans-anethole. Protein andvarious alkaloids are present in the seeds (Sahebkar *et al*., 2016; Hassanien *et al*., 2012; Zohary *et al*., 2012).

Drought is expected to continuously and significantly increase by the end of this century (Choat *et al*., 2012; Bu *et al*., 2018; Sun *et al*., 2020; Zabet *et al*., 2015; Bahamin *et al*., 2014). Water stress is problematic for plant growth and development (McDowell *et al*., 2011), as it limits access to the resources required for photosynthesis due to stomatal closure and the reduction of internal water transport (Breda *et al*., 2006). As such, water stress impairs normal plant functionality and further induces morphological, physiological, and biochemical changes to compensate for water limitations (Nassiri Mahallati *et al.*, 2022; Mitchell *et al*., 2013; Lee *et al*., 2016). Understanding the detailed patterns and mechanisms of responses by plants to water stress is central to predicting future plant functionality and resilience in the face of increasingly frequent drought episodes.

Drought is one of the most important abiotic stresses that reduces yield (Nassiri Mahallati *et al*., 2022). Researchers have stated that drought is a multidimensional stress that affects plants at different levels and affects almost all plant growth processes (Bahamin *et al*., 2013; Bahamin *et al*. 2014; Koocheki *et al*., 2017). An increase in the intensity of stress causes disturbance in physiological processes, growth stop and finally death of the plant due to water stress (Shekoofa & Emam, 2006). The effect of water stress on performance is multifaceted. In the stages of vegetative development, even a very minor stress can reduce the speed of leaf growth and in the later stages, the index of leaf area. The first apparent effect of water deficiency on plants is the smaller size and less number of leaves or plant height, which is caused by the decrease in cell development and growth, which is the most sensitive process affected by water stress. All these factors ultimately lead to a decrease in grain yield (Bahamin *et al*., 2019; Bahamin *et al*., 2021; Maleki *et al*., 2020).

The meta-analysis is a statistical methodology for the synthesis of results across multiple studies to attain an overall understanding of a given problem (Gurevitch *et al*., 2018). A recent meta-analysis has specifically addressed the responses of plants to drought stress (Dong *et al*., 2017); however it focused on the physiological indices (i.e., plant height, proline, electrolyte leakage, and root length) associated with transcription factors (C-repeat/dehydration-responsive element-binding proteins) that play important roles in plant response to environmental perturbations. Here we focus on the responses of ROS and enzymatic antioxidants (SOD, POD, CAT, GR, and APX), which represent the defense mechanisms of plants under abiotic stresses (Gill and Tuteja, 2010; Sun *et al*., 2019).

The purpose of meta-analysis is to obtain more information than the existing information, which is obtained by combining the results of smaller studies and with one or more statistical analyses. In this way, results that may have been discovered in smaller studies, using a meta-analysis of dozens of small studies (Matthews *et al*., 2012; Rotundo & Westgate, 2009).

Ren *et al* (2021) stated that in this study, we performed a meta-analysis to categorize the responses of physiological parameters involved in drought tolerance in (Aquaporin) AQP-overexpressing plants and to evaluate the experimental variables that affect transgenic plant performance. The results of various studies indicated that two primary physiological processes (osmotic adjustment and alleviation of oxidative damage) were significantly affected by AQP overexpression. Among the examined experimental variables, treatment media (soil), stress type (no watering), stress duration (long-term), recipient genus (Nicotiana), donor species (Musaceae), and gene family (PIP2) had positive impacts on drought tolerance in transgenic plants. These findings may help to guide future studies investigating the function of AQPs in the response of plants to water deficit stress. The results of the study of Sun *et al* (2020) showed that the results revealed that although water stress inhibited plant growth and photosynthesis, it increased reactive oxygen species (ROS), plasma membrane permeability, enzymatic antioxidants, and non-enzymatic antioxidants. Importantly, these responses generally increased with the intensity and duration of water stress, with a more pronounced decrease in ROS anticipated over time. Our findings suggested that the overproduction of ROS was the primary mechanism behind the responses of plants to water stress, where plants appeared to acclimatize to water stress, to some extent, over time. Our synthesis provides a framework for better understanding the responses and mechanisms of plants under drought conditions. The results of the study of Tahmasebi and Niazi (2021) showed that here, we used a combination of meta-analysis and network analysis to compare the transcriptional responses of Oryza sativa (rice), a C3 plant, and Zea mays (maize), a C4 plant, to drought stress.

Despite conducting numerous researches on drought stress on corn, it is impossible or difficult for researchers who study one or several related articles to make a precise summary and conclusion about reaching a definite conclusion. On the other hand, one of the limitations of planting most crops in Iran is the issue of drought stress, so Iran was chosen as the study area and meta-analysis was performed on the articles that have been worked on in Iran.

Therefore, the present study was conducted with the aim of reviewing, investigating and meta-analyzing the results of the researches conducted in the country with the aim of investigating the effect of drought stress on *Nigella sativa* production.

MATERIALS AND METHODS

data source

The research sample included the collection of articles from Syed's website, which found a total of 14 articles out of 60 articles related to the topic. The selection of articles was based on the studied traits, application of drought stress, variance analysis tables, and comparisons of treatment indices. And in this review, five attributes were investigated. These traits included seed yield, biological yield, percentage and yield of oil, percentage and yield per plant. To carry out these studies, the effect of drought stress on the quantitative and qualitative yield of black seed during the last 30 years (end of 2020) has been investigated. The articles were selected in such a way that the necessary data for meta-analysis were available in them. This information includes control treatment and stress treatments, standard deviation, experimental error and number of replicates (Hedges *et al*., 1999).

statistic analysis

The full description of meta-analysis statistical calculation method is provided by expert (Hedges *et al*., 1999; Gurevitch *et al*., 1999). The following steps are briefly described. The first step in meta-analysis is to calculate the difference between the control treatment and experimental treatments (stress treatment), which is called effect size (d). Therefore, a value is calculated for each of the 14 independent experiments that have been examined in this meta-analysis (Equation 1). It should be noted that the measured is specified for both the stress level and each stress level for evaluation.

Equation 1

$$d = \frac{\bar{X}_t - \bar{X}_c}{S_p} \times J$$

where, respectively $\bar{X}_c$ and $\bar{X}_t$ the mean of the control treatment and stress, Sp is the combined standard deviation of the means and J is the correction factor for the skewness of the mean deviation. J and Sp values are calculated from equations 2 and 3, respectively:

Equation 2

$$J = 1 - \left[\frac{3}{4(df_c + df_t) - 1}\right]$$

Equation 3

$$S_p = \sqrt{\frac{df_c({S_c}^2) + df_t({S_t}^2)}{df_c + df_t}}$$

in which Sc and St are respectively the standard deviation of the control and the stress treatment, dfc and dft are the degrees of freedom of the control and the stress treatment respectively. If the values of the standard deviation of the averages are not mentioned in the article, the value of Sp can be estimated based on the variance of the test error (MSE) that is presented in the analysis of variance tables in the articles from equation 4:

Equation 4

$$S_p = \sqrt{\left(\frac{n_c + n_t - 2}{n_c + n_t}\right) MSE}$$

where nc and nt are the number of control and treatment replicates, respectively.
Undoubtedly, all the tests under review do not have the same accuracy. Therefore, it is necessary to calculate a weight for each test according to its accuracy, and then to balance the effect size of each test with its help. For this purpose, first, the variance of the effect size for each experiment (Vd) was calculated (Equation 5):

Equation 5

$$V_d = \left[\frac{n_c + n_t}{n_c \times n_t}\right] + \left[\frac{d^2}{2n(n_c + n_t)}\right]$$

The opposite of this variance is the weight of that test, so any test with a smaller variance will have more weight: $w_i = \frac{1}{V_d}$

Finally, a total or cumulative effect size (d*) is calculated, which is actually the standardized difference between control and stress treatments for all the experiments under investigation (Equation 6):

Equation 6

$$d^* = \frac{\sum w_i d_i}{\sum w_i}$$

And its standard deviation (Sd*) will also be obtained from equation 7:

Equation 7

$$S_{d*} = \sqrt{\frac{1}{\sum w_i}}$$

The last stage of meta-analysis is the significance test of d*, knowing Sd*, the confidence interval of d* can be calculated. If this confidence interval overlaps with zero, the weighted cumulative effect size (d*) is not significant and the control and treatment are statistically different. otherwise, the difference between the treatment and the control is significantly greater than zero. In this study, in order to identify the relationship between the independent variable and the dependent variable, the correlation test and accumulation and funnel charts were taken with Excel software to obtain the relationships between the variables. A funnel plot or phenol is a graph that plots the effect size against the standard error of each study. If the corresponding funnel plot is asymmetric, this will cause a skewed distribution. Dissemination bias is a phenomenon that indicates that in meta-analysis data collection, statistically significant results are preferred over non-significant results. As a result of this issue, it causes uncertainty about the inference from the meta-analysis and its parameters should be corrected. The funnel plot is a method of correcting the diffusion bias in meta-analysis compared to the trim fill method. Researchers have presented a method that corrects meta-analysis parameters due to the problem of diffusion bias. First, the remote and asymmetric part of the funnel is removed after estimating the number of studies in this part, which is called trimming, then the remaining symmetrical part is used to estimate the true center of the graph, and finally, the removed studies are compared with the relative part. Those lost around the center are replaced, which is called filling (Duval & Tweedie, 2000).

Finally, the estimate of the overall mean as well as its variance are obtained based on the completed funnel plot. In meta-analysis, it should be investigated which one of the studies are in agreement with the null hypothesis and which one is against it, and for this purpose, accumulation or forest diagram is used. All calculations and graphs were done in Excel environment.

RESULTS AND DISCUSSION

Summary of the results of the tests under review

The results of this survey showed that from 1990 to 2020, 18 articles and theses have been published in the country regarding the impact of drought stress on the yield and quality of black seed. Out of this number, 18 papers had the control treatment or 100% of the crop capacity. 12 articles have treatments of 81-90% and 40-60% of the agricultural capacity of the farm and 14 articles have 61-80% of the agricultural capacity of the farm (Figure 1).

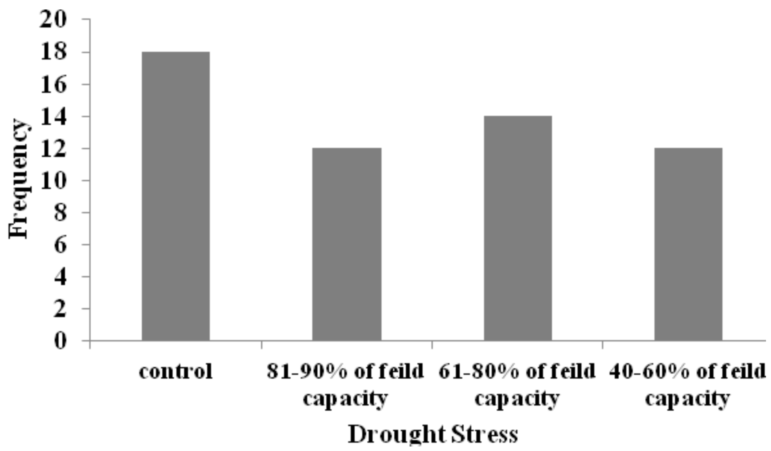

Figure 1- Distribution of the number of articles based on drought stress levels

Statistical comparison between drought stress levels

The effect size values for all 6 traits under investigation had a normal distribution, and the normality of this distribution is the main condition for the continuation of the meta-analysis calculations. Hedges *et al* (1999) stated that if the distribution of the effect size values is not normal, the logarithm of the mean difference of the control and experimental treatments should be used to perform meta-analysis.The standardized values of the effect of drought stress on grain yield and dry matter yield were significant ($P < 0.001$). It is reminded that the effect size for each trait is the difference between the average drought stress treatment and the average of the control treatment, so its positive values indicate that the average treatment is higher than the control.

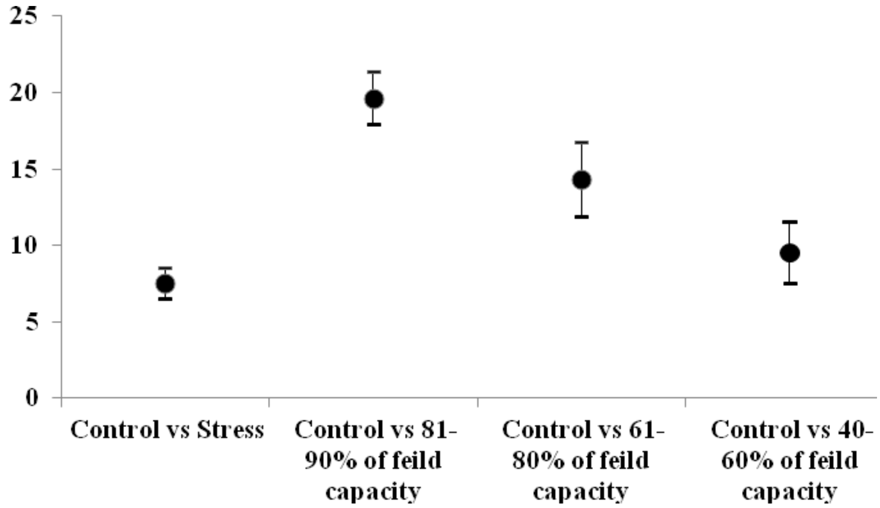

Figure 2- Comparison of the effect of different levels of drought stress on grain yield. The vertical lines are the confidence intervals of the weighted cumulative effect size across the trials under investigation. The first comparison is the control versus the mean of stressed treatments.

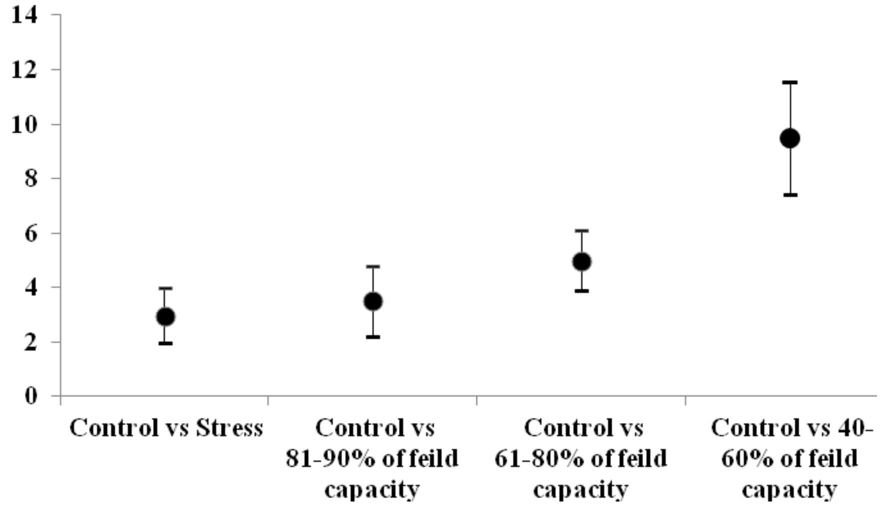

Figure 3- Comparison of the effect of different levels of drought stress on biological performance. The vertical lines are the confidence intervals of the weighted cumulative effect size across the trials under investigation. The first comparison is the control versus the stressed mean.

Regarding the percentage of oil and percentage of protein (Figures 4 and 5), the results indicate that the average effect size does not have a significant difference with the control treatment, and therefore the drought stress treatments did not have an effect on the significant increase or decrease of oil percentage and protein percentage compared to the control. Is. In different experiments, different results of the effect of drought stress on the percentage of oil and protein have been mentioned, so that in some of them, the percentage of oil and protein has decreased under the influence of stress, and in others, the percentage of oil and protein has increased under the influence of increasing the intensity of drought stress. Is. These cases have caused an increase in data dispersion and the non-significance of these attributes.

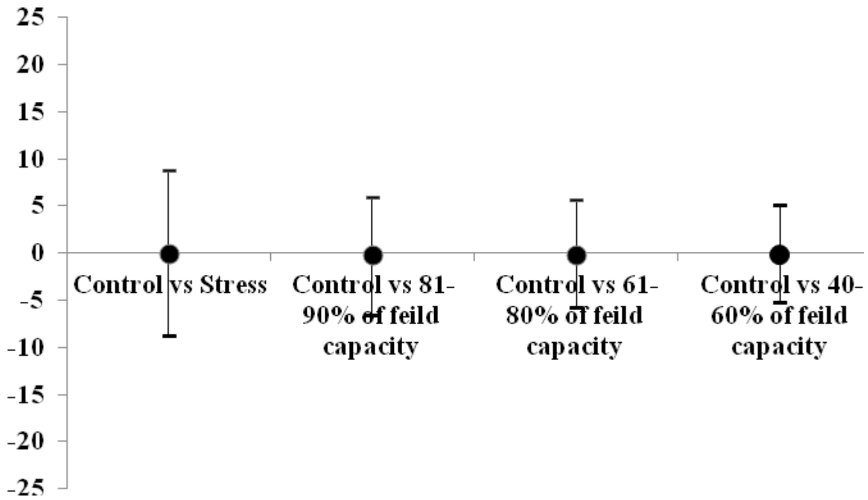

Figure 4- Comparison of the effect of different levels of drought stress on oil percentage. The vertical lines are the confidence intervals of the weighted cumulative effect size across the trials under investigation. The first comparison is the control versus the stressed mean.

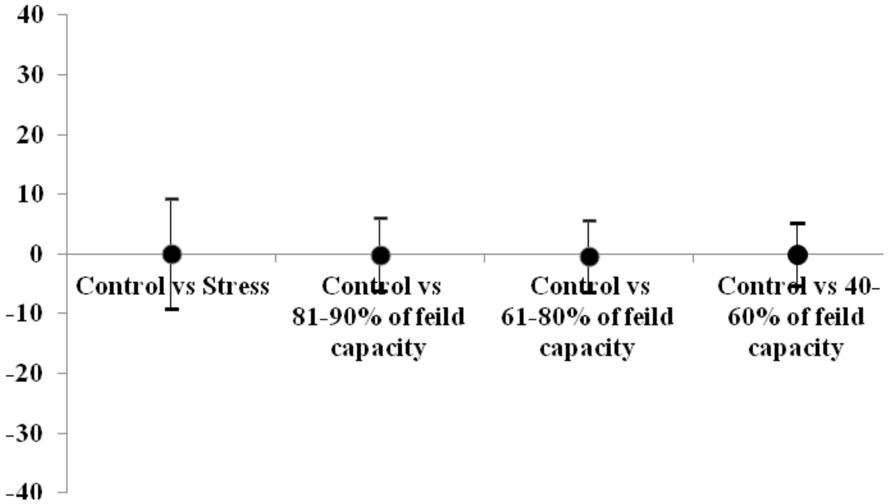

Figure 5- Comparison of the effect of different levels of drought stress on protein percentage. The vertical lines are the confidence intervals of the weighted cumulative effect size across the trials under investigation. The first comparison is the control versus the mean of stressed treatments.

The standardized values of the effect of drought stress on oil yield were significant ($P < 0.001$) when comparing the control treatment with the overall drought stress treatment and the control treatment with 40-60% of the field's agricultural capacity ($P < 0.001$), but there was a statistically significant difference between the control treatments. It was not significant with 81-90 and control with 61-80 percent of the agricultural capacity of the farm. In addition to oil percentage, oil yield is affected by grain yield, since grain yield was significantly affected by drought stress and its value decreased with increasing stress intensity; The yield of oil also decreased due to drought stress. The effect size for each trait is the difference between the average of the drought stress treatment and the average of the control treatment, so its positive values indicate that the average of the stress treatment is higher than the control (Figure 6).

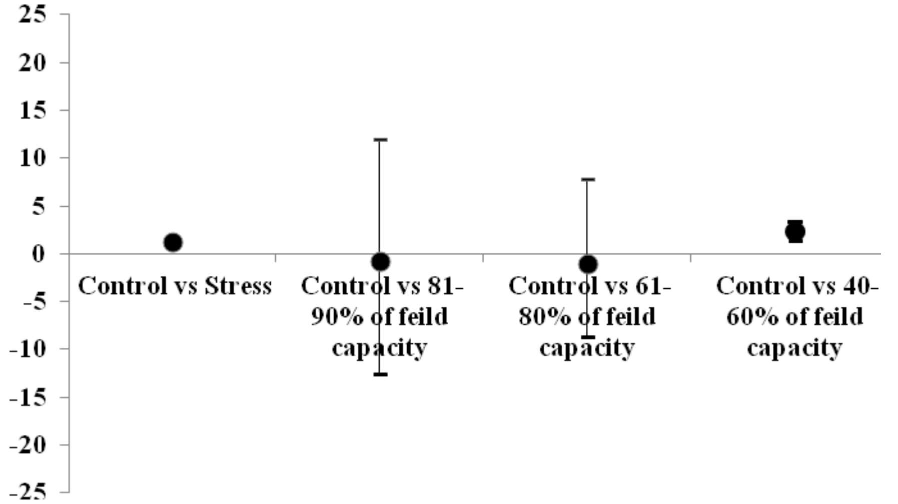

Figure 6- Comparison of the effect of different levels of drought stress on oil yield. The vertical lines are the confidence intervals of the weighted cumulative effect size across the trials under investigation. The first comparison is the control versus the mean of stressed treatments.

The standardized values of the effect of drought stress on protein yield were significant (P < 0.001) when comparing the control treatment with the overall drought stress treatment and the control treatment with 40-60% of the field's agricultural capacity (P < 0.001), but there was a statistically significant difference between the control treatment. It was not significant with 81-90 and control with 61-80 percent of the agricultural capacity of the farm. In addition to protein percentage, protein yield is also affected by grain yield, since grain yield was significantly affected by drought stress and its value decreased with increasing stress intensity; The yield of oil also decreased following drought stress (Figure 7). Therefore, the significant increase in protein and oil yield under the influence of drought stress can be justified.

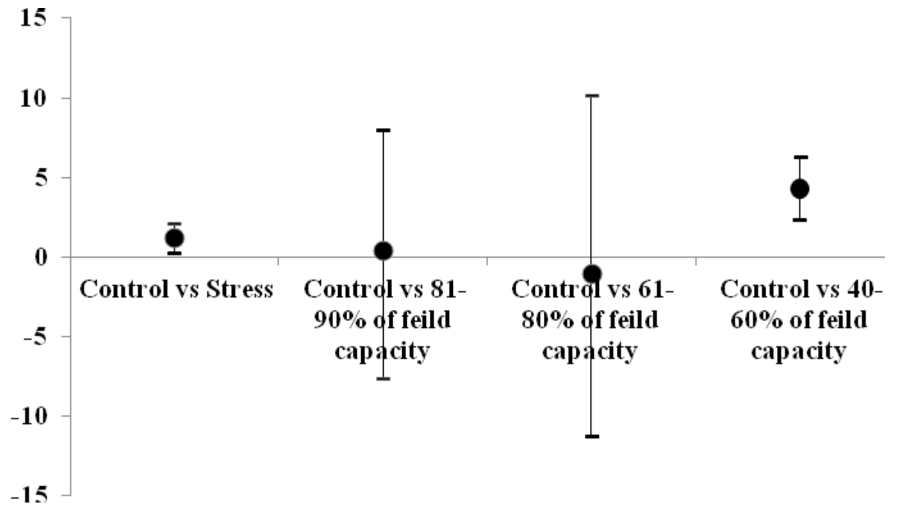

Figure 7- Comparison of the effect of different levels of drought stress on protein yield. The vertical lines are the confidence intervals of the weighted cumulative effect size across the trials under investigation. The first comparison is the control versus the stressed mean

Nigella response to drought stress

Combining the data related to all the experiments under review showed that the reaction of black seed yield to drought stress follows the 1st form (Figure 8). The grain yield decreased linearly with the increase of drought stress up to 40% of the agricultural capacity of the field. The coefficient of explanation between drought stress and black seed yield was 0.46 and the correlation results also showed that the relationship between these two dependent and independent variables was significant.

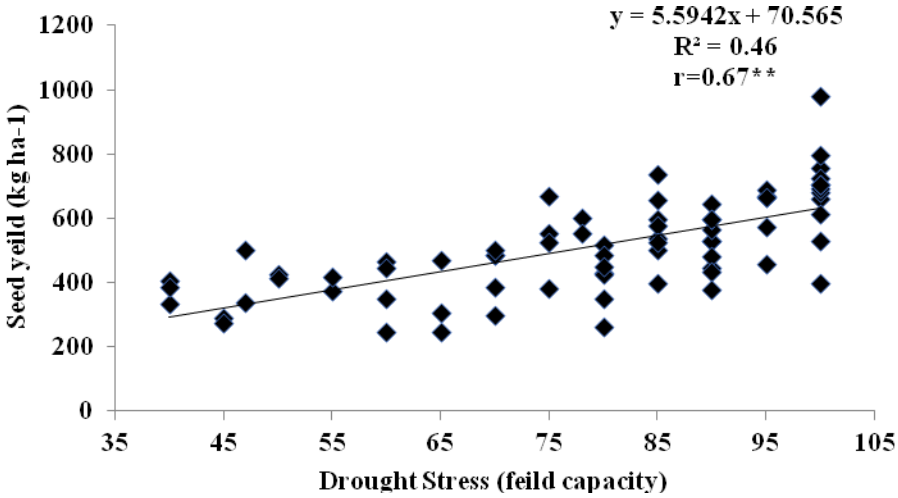

Figure 8- Grain yield response to drought stress levels in the experiments under investigation, the significance of the explanatory coefficients was determined based on the Pearson table.

Wheat dry matter yield also showed a similar response to grain yield to drought stress, although compared to yield; Its effectiveness was higher because the regression was 0.58 and the correlation results also showed that the relationship between drought stress and total dry matter was significant and with increasing drought stress; The biological performance of nigella decreased (Figure 9).

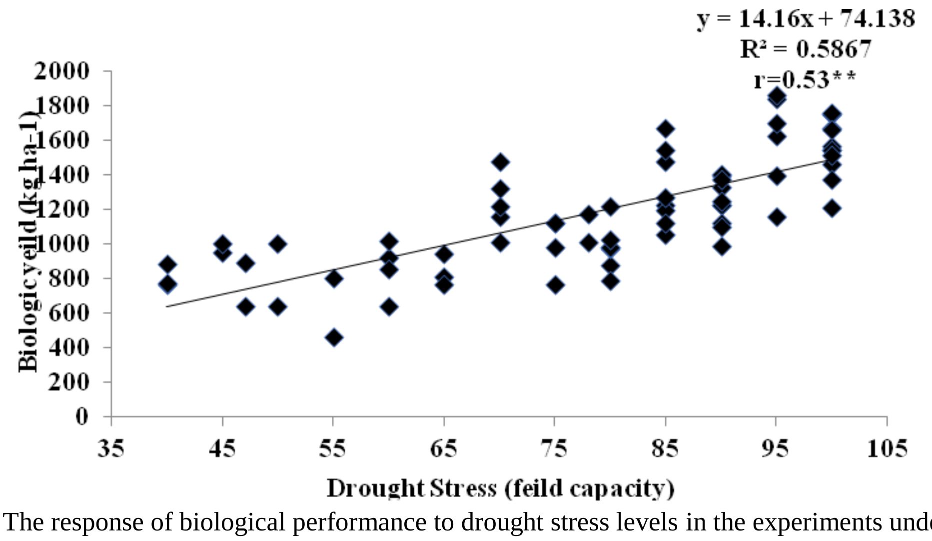

Figure 9- The response of biological performance to drought stress levels in the experiments under review, the significance of the explanatory coefficients was determined based on Pearson's table.

The regression results showed that there was a weak and non-significant relationship between oil percentage and protein percentage with drought stress. The correlation results also showed that the relationship between drought stress and oil percentage (0.14) and protein percentage (0.22) was not significant (Figures 10 and 11). Probably, many environmental and genetic factors have neutralized the effect of drought stress on the percentage of black seed oil and protein.

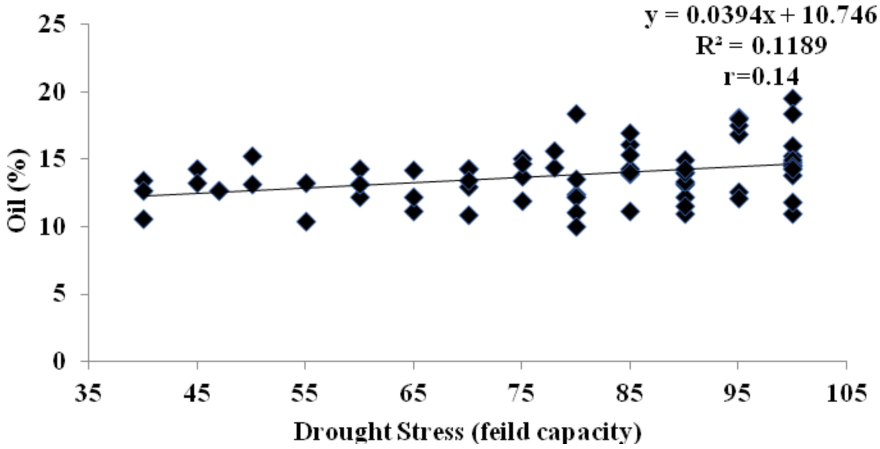

Figure 10- The reaction of oil percentage to drought stress levels in the experiments under investigation, the significance of the explanatory coefficients was determined based on the Pearson table.

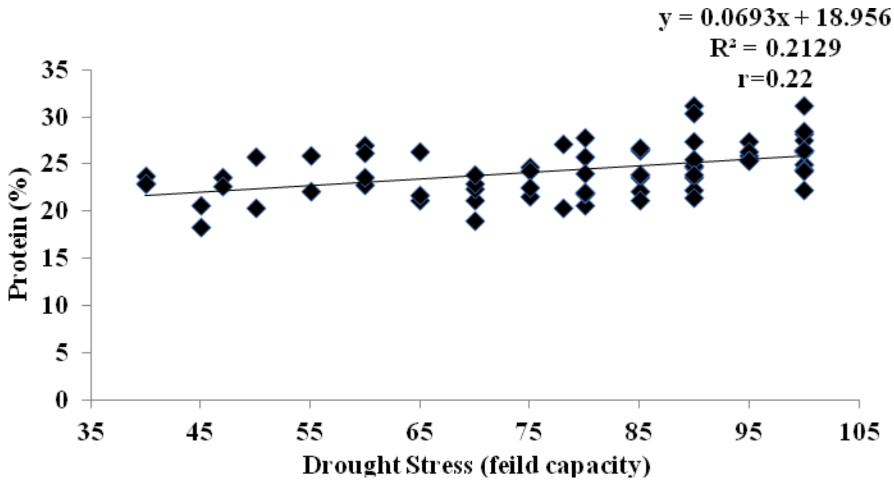

Figure 11- The reaction of protein percentage to drought stress levels in the experiments under investigation, the significance of the explanatory coefficients was determined based on the Pearson table.

The yield of oil and protein decreased linearly with the increase of drought stress up to 40% of the agricultural capacity of the farm. The coefficient of explanation between drought stress and oil yield and black seed protein yield was 0.41 and 0.63, respectively, and the correlation results also showed that the relationship between drought stress with oil percentage and protein percentage was 0.42 and 0.44. It was significant (Figures 12 and 13). The yield of oil and yield of protein were affected by two factors, the percentage of the material itself and grain yield, since grain yield was affected by drought stress; Oil yield and protein yield were significantly affected by drought stress.

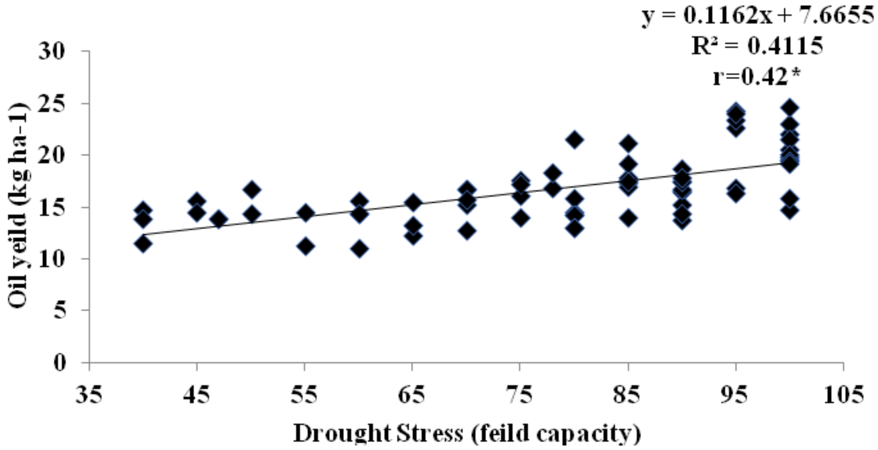

Figure 12- The reaction of oil yield to drought stress levels in the experiments under investigation, the significance of the explanatory coefficients was determined based on the Pearson table.

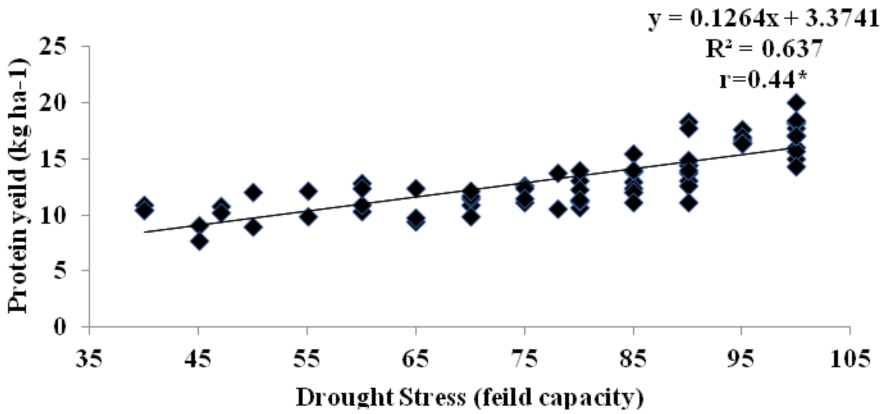

Figure 13- The reaction of protein yield to drought stress levels in the experiments under review, the significance of the explanatory coefficients was determined based on the Pearson table.

An increase in the intensity of stress causes disturbances in physiological processes, stopping growth and eventually death of the plant due to water stress. The effect of water stress on performance is multifaceted. In the stages of vegetative development, even a very minor stress can reduce the speed of leaf growth and in the later stages, the index of leaf area. The first apparent effect of water deficiency on plants is the smaller size and less number of leaves or plant height, which is caused by the decrease in cell development and growth, which is the most sensitive process affected by water stress. All these factors ultimately lead to a decrease in grain yield (Hissao, 1973). The decrease in grain yield as a result of drought stress and increased irrigation intervals has also been reported by Ohashi et al (2009).

Behnamnia et al (2009) by studying the mutual effect of drought stress on fresh and dry weight of spruce plants, showed that lack of water causes a decrease in fresh and dry weight of the stem. The dry weight of aerial parts has a significant correlation with the number of sub-branches, plant height, number and surface of leaves and durability of leaf surface. Therefore, the effects of drought on the leaf can be mentioned that the growth and development of the leaf is affected even with a very small decrease in humidity, before the photocenter is reduced. In fact, the most important result of the sensitivity of cell growth to lack of moisture is a significant reduction in leaf growth and as a result, leaf area. With the reduction of leaf area and reduction of phytosynthesis, the biological performance of the plant decreases. The researchers showed that water stress had a significant effect on the performance of the flowering branch, and the highest dry matter yield was observed in the full irrigation treatment and the highest oil percentage of the flowering branch was observed in the severe stress treatment. The slope of dry matter reduction with increasing stress intensity was observed in the present study. The stress causes the plant to not go through the vegetative stage well and enter the reproductive period with weak potential, therefore, reducing the amount of produced assimilate, in addition to preventing the elongation of the stem and reducing the number of leaves and axis of the inflorescence, limits the formation of a large number of flowers and follicles. and in other words, the capacity of the reservoir and the production capacity of the source are both reduced. With the increase of salinity stress, the ionic toxicity resulting from the increase of harmful elements, which causes disturbance in all biological and metabolic activities of plants, ultimately leads to the loss or severe reduction of aerial organs.

Bahamin *et al* (2021) reported a decrease in the amount of protein in black seed under drought stress. The reduction of protein amount is a common phenomenon in stresses such as drought stress, and drought stress suppresses the production of some proteins and induces the synthesis of new proteins. Also, the oxidative stress and oxidation of proteins and as a result the increase of oxidized proteins, the suppression of protein synthesis through the effect on poly-rhizobiomes and the increase of proteolysis of proteins, the reduction of the efficiency of the mechanisms involved in the repair and regeneration of proteins. and reduction of nitrate reductase enzyme activity have been mentioned as reasons for the reduction of protein amount in drought stress.

Banayan *et al* (2008) in the study of different irrigation treatments on black seed quality concluded that black seed is tolerant to water deficit except during seed formation. The lowest seed yield occurs when irrigation is stopped during flowering. And the number of seeds per plant is the main factor that is affected at this time. The results also show that oil concentration is not affected, but oil yield is significantly affected by drought stress.

CONCLUSION

The findings of this research showed that despite many studies regarding the effect of drought stress on black seed yield in the country, the results are very diverse and widely scattered. This issue has caused the exact effect of drought stress, especially on the percentage of black seed oil and protein, to be unknown. Meta-analysis, as a powerful statistical method, provided a suitable and accurate tool for combining the results of independent experiments and established specific ranges for the effect of drought stress, especially on the quantity of black seed. In this study, it was found that although drought stress did not have a significant effect on the percentage of oil and protein, it caused a decrease in oil yield and protein content of black seed. Drought stress has harmful effects on the performance of black seed, and it is necessary to think of appropriate solutions to reduce the effect of drought stress on the quality of this valuable medicinal plant.

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