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Meta-Analysis of Studies on the Effect of Nitrogen Fertilizer, Water Stress, And Density on the Quantitative and Qualitative Characteristics of Peppermint (Mentha Piperita L.)

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Abstract: - How to bring together and interpret independent research studies is a fundamental question in all sciences. Using the meta-analysis technique allows researchers to gain more knowledge about the desired phenomena compared to a single or single study or intervention. In the past years, many studies have been conducted on the peppermint plant, which sometimes have contradictory results. Twenty English studies and 37 Persian research were chosen from a total of 250 studies following quality control. Lastly, a meta-analysis was conducted on 36 papers that included 283 effect sizes for water stress, chemical fertilizer (Nitrogen), and various planting densities on the quantitative and qualitative characteristics of peppermint. The impact of chemical fertilizers (Nitrogen) on the quantitative characteristics of biomass, dry weight, and plant height as well as the qualitative characteristics of oil yield, oil percentage, and oil constituents like menthone and limonene is shown in the meta-analysis results to be significant ($P \geq 0.01$). The menthol ingredient was negatively impacted by nitrogen chemical fertilizer, with no discernible effect ($g = -0.408$). The studies of the effects of chemical fertilizers (Nitrogen) on the studied traits have all been heterogeneous and asymmetric and confirm the use of the random model. Only the studies of the ingredients of the essential oil, i.e. limonene and linalool, are symmetric and show no diffusion error. Other studies require replication of studies for recommendation different planting densities have a considerable impact on all quantitative and qualitative features, with a rather homogenous and symmetric effect. ($P<0.05$). The plant's height yielded the largest effect size ($g=1.108$), while the oil percentage ($g=0.564$) produced the average effect size. Aside from oil percentage, other features are also significantly impacted by the heterogeneous and asymmetric effect of water stress. ($P<0.05$). An increase in the amount of essential oil is positively correlated with water stress. Furthermore, although studies do not demonstrate propagation mistakes, they have had a noteworthy and adverse impact on quantitative features. However, proline levels are positively and significantly impacted by water stress, and this effect is symmetrical. ($P<0.05$). The largest amount of positive effect obtained was on the amount of proline ($g = 1.337$) and the largest negative effect was related to plant height ($g = -1.357$). Meta-analysis allows us to examine and calculate its direction and strength with a more detailed and deeper investigation in addition to estimating the amount of impact.

Keywords: Effect size, Diffusion error, Heterogeneity, Irrigation

I. INTRODUCTION

How to bring together and interpret independent research studies is a fundamental question in all sciences. The inadequacy of the results of a single study and the need to combine the findings by scientists have led to the development of methods that allow combining the results of many independent studies (Anjomani et al., 2022). After the 1980s, scientists began to develop statistical methods or meta-analysis, and thus, meta-analysis became a statistical technique (Çoğaltay & Karadag, 2015). In the meta-analysis method, it is possible to present single and reliable results to other researchers by collecting data and comparing the findings of various studies and research conducted in one of the scientific-applied fields. gave (Viechtbauer, 2010). Meta-analysis is the statistical analysis of a large set of statistical results related to different studies to integrate its findings (Izanlo & Habibi, 2011). In this case, results that may not be obtained in smaller studies will be obtained using a meta-analysis of dozens of small studies (Rotundo & Westgate, 2009). For many centuries, the active ingredients or secondary metabolites found in medicinal plants have been used as partial sources to develop novel and cutting-edge medications. The results showed that the integrative application of 50% chemical fertilizer + nano-chelated fertilizer (in the first harvest) and control conditions (in the second harvest) respectively produced the highest and lowest growth parameters, including plant height, number of lateral branches per

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plant, and leaf greenness (SPAD index). Also, the maximum concentration of N, P, K, and Fe was reached in the first harvest with the application of 50 % chemical fertilizer + nano-chelated fertilizer. Furthermore, the highest peppermint dry matter yield (354.8 g/m2), oil %content (2.7 %), and oil %yield (6.6 g/m2) were achieved at the first harvest with the application of 50 % chemical fertilizer + nano chelated fertilizer (Maggi, et al. 2020). The results indicated that N levels affected plant growth and chlorophyll content. The main constituents of the oil %were carvone, limonene, 1,8-cineole, germacrene D and -pinene, the recommendation for increasing upper plant biomass and oil %quality in spearmint is to employ 200 mg/L N (Tzortzakis, et al 2017)

In examining the effect of irrigation and zinc nutrition on the growth and antioxidant properties of the medicinal plant peppermint (Menta piperitaL.), the results of the experiment showed that the effect of irrigation on leaf length, leaf width, number of leaves, diameter of the main stem, number of internodes, number of secondary branches, Plant height, fresh weight and dry weight of stem, fresh weight and dry weight of leaf and fresh and dry weight of plant were significant at 1% probability level. To increase the effectiveness of peppermint medicinal plant essential oil, 100 humidity treatment and 2.5 mg zinc foliar spray should be used (Nejatzadeh et al., 2015). In the investigation of the effect of urea chemical fertilizer and naproxen biological fertilizer, the results showed that it has an effect on the quantitative and qualitative traits of peppermint and it is significant that the highest dry weight yield is 550 kg per hectare and the percentage and yield of oil %is 3.7% and 2.35 kg per hectare, respectively. The user used 200 kg of urea along with Nitroxin biofertilizer (4 kg per hectare) (Dehaghi et al., 2014).

It does not influence leaf oil percentage, but it does alter plant density, wet and dry weight yield, plant oil percentage, and oil yield. At a density of 20 plants per square meter and a rate of 21.15 liters per hectare in the second year, the maximum oil yield percentage was achieved. (Heidari et al., 2008). An experiment was conducted on different levels of density and different levels of Nitrogen amounts and the results showed that the use of 10 plants per square meter and 168 kg per square meter had a significant effect on all quantitative traits. Pure Nitrogen per hectare was found to be the best treatment for pepper production (Shirani-Rad et al., 2008). The highest yield of wet and dry leaf weight was obtained at a density of 6 plants per square meter. By increasing the density up to 30 plants per square meter, the yield index of fresh weight decreased by up to 70%. At the density of 6 plants per square meter, the highest yield of oil %was obtained at the rate of 2.25%. The results showed that the leaf yield increases in low density and the amount of oil %increases in medium density (Amani and Elfati, 2016). Due to the existence of many differences in the selection of the population, sample, independent and dependent variables, statistical methods used in studies, and experimental designs, it seems difficult to compare studies and obtain a coherent and coordinated result from them. This situation becomes more difficult when the number of studies increases day by day and sometimes the results obtained from them are very contradictory (Habibi et al., 2009). Meta-analysis is a research method for answering questions about the results of previous studies, questions that a single study cannot answer. The combination of study data and the comparison of studies are the two main foci of meta-analysis. Additionally, it uncovers fresh findings from earlier investigations, opens up fresh avenues for inquiry, and might even be able to calculate the ideal sample size for upcoming studies (Cooper et al., 1994). In such a situation and considering the importance of medicinal plants, especially the increase in the cultivated area of peppermint plants, this research by collecting studies on chemical fertilizer (Nitrogen), water stress and irrigation, and different planting densities and using a comprehensive technique The meta-analysis was conducted to examine the recorded effects in wider dimensions and the results of the meta-analysis, which has higher power, orientation, and precision, were examined along with the declaration of the presence or absence of publication errors of the conducted studies. Along with the announcement of the presence or absence of publication errors, the conducted studies were reviewed and guidance was given in the fields needed by the researchers.

II. MATERIAL AND METHODS

To perform this meta-analysis, the method introduced by Cooper was used. This method has the following steps: searching and selecting studies (data collection), coding the characteristics of each study, extracting data suitable for meta-analysis, and analyzing the data. In this research, standardized mean difference and correlation coefficient were used. It has been used to determine the size of the Hedges effect (g). The standardized mean difference method and the correlation coefficient to determine the Hedges effect size value (g) have been used in the meta-analysis method for the effect sizes in this research since the intended benefit in this meta-analysis is the difference between the averages of the treatment group (experiment) and the control group (control) (Pouresmaeily et al., 2011). The standard difference between the mean of the control treatment and the mean of the experimental treatment is called the effect size (g) Equation (3)and (4). It was used in performance between control and experimental treatments (Cohen, 1988).

$$(3)\ g = \frac{M_1 - M_2}{S_{pooled}}$$

In this regard, M1and M2 is the average performance of experimental treatment and control treatment and S_{Pooled}, standard deviation, respectively (Cohen, 1988).

$$(4)\ s_{pooled} = \sqrt{\frac{(n1-1)s1^2+(n2-1)s2^2}{n1+n2-2}}$$

In this regard, S1 and S2 are respectively the square root of the integrated variance of two treatment groups.

If there is heterogeneity between studies, the random effect model is used. I^2index is one of the important indices in determining the degree of heterogeneity between studies.

In general, the degree of heterogeneity in the I^2index is determined in the following three ways:

$I^2 \leq 25\%$ indicates low heterogeneity.

$50 \leq I^2 \leq 75\%$ indicates moderate heterogeneity, that is, half of the total variability among effect sizes is not due to sampling error and is due to heterogeneity between studies.

And $I^2 \geq 75\%$ indicates high heterogeneity (Higgins et al., 2003).

All the extracted data were transferred to comprehensive meta-analysis software for meta-analysis. The results reported about each of the variables were analyzed using comprehensive meta-analysis software (CMA. Version 2), developed in the United States.

III. RESULTS AND DISCUSSION

From a total of 57 selected studies (37 domestic studies and 20 foreign studies), the data of 36 studies were capable of meta-analysis, and finally, there were 283 effect sizes for chemical fertilizers, 107 effect sizes for water stress, 98 effect sizes for density, and on dry weight, essential oil percentage, and oil yield of peppermint were recorded and prepared for meta-analysis and the rest of the studies were excluded.

Meta-analysis of studies on the effects of different amounts of chemical fertilizer (Nitrogen) on the quantitative and qualitative characteristics of peppermint plants.

In the studies on the effect of chemical fertilizers (Nitrogen) also for dry weight traits 44 effect size, essential oil percentage 45 effect size, plant height 52 effect size, oil yield 45 effect size, biomass 7 effect size, limonene 17 effect size, linalool 12 effect size, menthol 19 effect sizes, menthone 19 effect sizes, penine 19 effect sizes and proline 4 effect sizes have been calculated (Table 1).

Null hypothesis test

In the random effects model in meta-analysis studies, the null hypothesis is based on the fact that the average size of the real effects of all studies is zero. Based on this, the null hypothesis is confirmed or rejected based on the z value and its statistical significance (Vahedi, 2021).

The results (Table 1) show that the effect of chemical fertilizers (Nitrogen) on the quantitative traits of biomass, dry weight, plant height, and the qualitative traits of essential oil percentage and oil yield is significant, and essential oil percentage constituents such as limonene and menthone are also significant. Is ($P \geq 0.01$). But it is not significant on other ingredients of essential oil % such as menthol, linalool, penine and on the amount of proline. The largest effect size obtained on quantitative traits was obtained on biomass trait (g = 1.318) and qualitative traits, the highest effect size was obtained for Menthone (g = 0.962). Nitrogen chemical fertilizer had a negative and non-significant effect on menthol substance (-0.408 g).

Table 1- Effect size of different amounts of fertilizer (Nitrogen) on quantitative and qualitative traits of peppermint with 95% confidence level

Random model	Number	g effect size	Standard error	Variance	Low limit	High limit	Z value	P value
Chemical fertilizer- essential oil %	45	0.721	0.145	0.021	0.437	0.005	4.975	0.000
Chemical fertilizer-dry weight	44	0.867	0.129	0.017	0.614	1.121	6.708	0.000
Chemical fertilizer - height	52	0.870	0.111	0.012	0.652	1.087	7.828	0.000
Chemical fertilizer-oil yield	45	0.878	0.122	0.015	0.639	1.118	7.184	0.000
Chemical fertilizer - biomass	7	1.318	0.248	0.061	0.832	1.803	5.321	0.000
Chemical fertilizer- Limonene	17	0.824	0.354	0.125	0.131	1.517	2.331	0.020

Chemical fertilizer-Linalool	12	0.235	0.421	0.178	-0.591	1.060	0.557	0.578
Chemical fertilizer-Menthone	19	0.962	0.286	0.082	0.402	1.522	3.369	0.001
Chemical fertilizer-Menthol	19	-0.408	0.345	0.119	-1.085	0.269	-1.181	0.238
Chemical fertilizer-Pennine	19	0.584	0.401	0.161	-0.202	1.369	1.456	0.145
Chemical fertilizer-Proline	4	0.642	0.645	0.417	-0.623	1.907	0.995	0.320

Cohen's comparison table can be used to interpret the effect size values (Table 2). Based on the size of the obtained effects, the effect of chemical fertilizer (Nitrogen) on quantitative and qualitative traits is positive and is in the high and high range, but on the components and content of the essential oil, The degree of influence varies, ranging from limonene's growing and positive effect to menthol's negative and mild effect. Furthermore, Menthone varies. Consequently, varying levels of artificial fertilizer (Nitrogen) have a significant impact on both the quantitative and qualitative performance.

Table 2. Effect size interpretation based on Cohen's scale

Random model	Effect size
Low	0.2
Moderate	0.5
High	0.8

The Forest plot of the effect of chemical fertilizer on the ingredient of essential oil % (menthol) showed, it can be seen that there are studies on both sides of the zero axis and a large number of studies have crossed the zero line, which shows the insignificant effect of chemical fertilizers on menthol. Significant tree diagrams were not drawn in other traits.

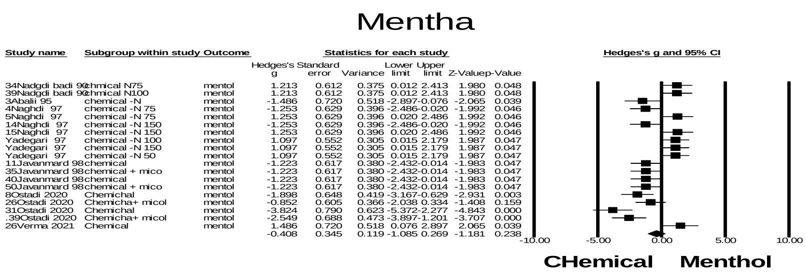

Figure 1 The Forest plot of the effect size of chemical fertilizer (Nitrogen) on peppermint Menthol

Heterogeneity test

The heterogeneity test examines the difference between the effect sizes of the studies and examines the null hypothesis that all the studies examined in a meta-analysis have the same effect size. As shown in (Table 3). Heterogeneity can be due to the diversity of studies, sampling error, type of research design, sample size, etc., in this research, the high heterogeneity between effect size studies fully justifies the use of a random model.

I^2index is one of the important indices in determining the level of heterogeneity between studies. In contrast to other indices, this one's accuracy is proportional to heterogeneity, or the overall variance among research, and is not influenced by the quantity of studies. It demonstrates that only 49% of the variance is attributable to random error, and only 0.61% of the observed heterogeneity between study effect sizes is due to real differences in effect sizes as a result of the influence of the intervention or research design. This value for oil yield is equal to I^2= 45.819, for dry weight I^2= 50.957, and for limonene amount I^2= 80.446. which shows the high heterogeneity between the studies and the obtained results in this attribute.

One of the causes of heterogeneity in the results of studies may be the difference in research design and sample size. Studies with smaller sample sizes are more likely to produce larger effect sizes than studies with larger sample sizes.

Studies with larger sample sizes have more accurate effect size estimates compared to studies with smaller sample sizes. In addition, small studies with non-significant effects may never be published or even published.

Table 3 shows the results of Bag and Mazumdar's heterogeneity test. Statistical tests should be used to ensure the correct result of the funnel diagram and to determine the propagation error. In studies that are asymmetric for recommendation and implementation, it is better to conduct more studies, but symmetric studies ($P \leq 0.05$), do not have publication errors, and the meta-analysis results can be considered correct and recommendable with 95% confidence. . The results show that the studies of the effect of chemical fertilizers (Nitrogen) on the studied traits were all heterogeneous and asymmetric and confirm the use of the random model. Only two groups of studies of essential oil percentage ingredients, namely limonene and linalool, show symmetry and no diffusion error. According to the result of the null hypothesis test, it can be said that the effect of chemical fertilizer on the increase of limonene substance is positive and significant has no diffusion error, and is recommended. Other studies have publication bias. Which needs to conduct and repeat more studies for recommendation.

Table 3- Test of heterogeneity of different amounts of chemical fertilizer (Nitrogen) on quantitative and qualitative traits of peppermint plant with 95% confidence level

Traits	I-squared	Z Z value	P P value	Test result
Chemical fertilizer - biomass	0.000	2.252	0.024	Heterogeneous symmetric
Chemical fertilizer-dry weight	50.957	6.169	0.000	Heterogeneous symmetric
Chemical fertilizer - height	44.084	5.129	0.000	Heterogeneous symmetric
Chemical fertilizer-oil yield	45.819	4.803	0.000	Heterogeneous symmetric
Chemical fertilizer essential oil %	61.544	5.438	0.000	Heterogeneous symmetric
Chemical fertilizer-Limonene	80.446	1.771	0.076	Homogeneous asymmetric
Chemical fertilizer-Linalool	81.473	1.371	0.170	Homogeneous asymmetric
Chemical fertilizer-Menthol	74.414	2.763	0.005	Heterogeneous symmetric
Chemical fertilizer-Pennine	84.089	2.471	0.013	Heterogeneous symmetric
Chemical fertilizer-Proline	74.968	0.500	1.000	Heterogeneous symmetric

Publication error

Drawing a funnel plot is used as a valuable method to detect any diffusion bias or diffusion error in meta-analysis studies. In this diagram, the simple distribution shows the effect size of each study around the mean axis. Usually, the effect size index is placed on the horizontal axis and the effect size dispersion is placed on the vertical axis. In an ideal case where there is no publication error, the chart design represents a symmetric inverted funnel. The effect of smaller studies is more widely scattered at the bottom of the chart and larger studies are more accurately placed at the top of the funnel. The asymmetry of the funnel diagram indicates the existence of the publication error, which means that small studies that had a large and significant effect size had a greater chance of being published, and small studies that had a small or non-significant effect size were ignored. Funnel diagram, studies It shows the effect of chemical fertilizer (Nitrogen) on limonene in peppermint essential oil. Most of the studies are located in the middle and bottom of the funnel, which indicates studies with small data volumes that show a larger effect size. In the studies of Nitrogen chemical fertilizer, it has become significant on all qualitative and quantitative traits Only for two substances, limonene, and linalool, there is symmetry and no bias in distribution, and it is recommendable, and other traits need to be repeated more studies. The middle and inside are inverted and it can be said that these studies do not have a publication error and the effect size values are acceptable with 95% confidence (Figure 2).

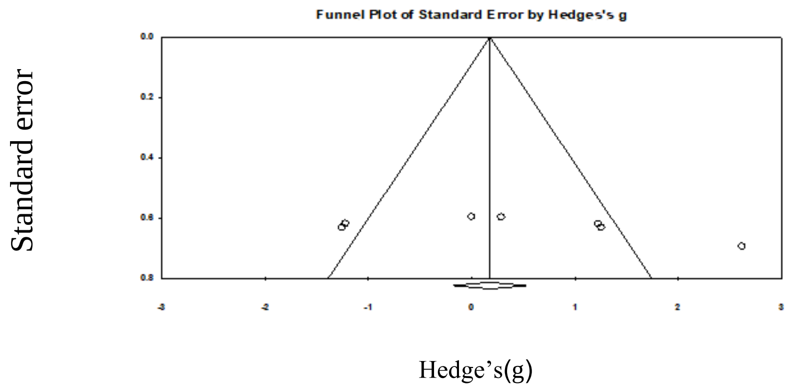

Figure 2 The Funnel plot of the effect of chemical fertilizer (Nitrogen) on peppermint Limonene

Meta-analysis of studies on the effect of density and water stress on the quantitative and qualitative characteristics of peppermint plants

The results (Table 4) show different planting densities have been significant on all quantitative and qualitative traits. (P≥0.01). The effect on dry weight and height is high and the percentage of oil %is moderate. The size of the effect obtained on the height of the plant is the highest (g = 1.108) and the average effect size on the essential oil % is obtained (g = 0.564). The effect of water stress other than the essential oil % on other traits is significant (P≥0.01). Water stress has a positive effect on increasing the percentage of essential oil, but it has a significant and negative effect on quantitative traits (dry weight, plant height, and oil yield). However, water stress has a positive and significant effect on the amount of proline (0.01). ≥P). The largest amount of positive effect obtained was on the amount of proline (g = 1.337) and the largest negative effect obtained was related to plant height (-1.357 g).

Table 4- Effect size of density and water stress on quantitative and qualitative traits of peppermint plant with 95% confidence level

Random model	Number	g effect size	Standard error	Variance	Low limit	High limit	Z value	P value
Density-essential essential oil %	29	0.564	0.167	0.028	0.326	0.981	3.909	0.000
Density-dry weight	29	0.823	0.436	0.190	-2.48	-0.77	-3.726	0.000
Density-height	11	1.108	0.248	0.062	0.621	1.595	4.560	0.000
Density - yield	29	0.826	0.141	0.020	0.551	1.102	5.872	0.000
Water tension – essential oil %	34	0.050	0.218	0.048	-0.378	0.477	0.228	0.819
Water stress-dry weight	32	-1.155	0.110	0.012	-1.370	-0.940	-10.523	0.000
water stress- height	13	-1.357	0.186	0.035	-1.721	-0.992	7.298	0.000
Water stress-oil yield	21	-0.901	0.205	0.042	-1.302	-0.500	-4.403	0.000
Water stress-proline	7	1.337	0.250	0.063	0.846	1.828	5.337	0.000

Figure 3- The Forest plot shows the effect of water stress on the percentage of essential oil. In the studies of water stress (Tention), almost half of the studies had a positive effect and the other half had a negative effect, which shows that water stress or tention has a positive and small effect on the increase of the essential oil percentage with any amount, and of course for the reason mentioned and considering cutting the zero-axis line. This effect is not significant.

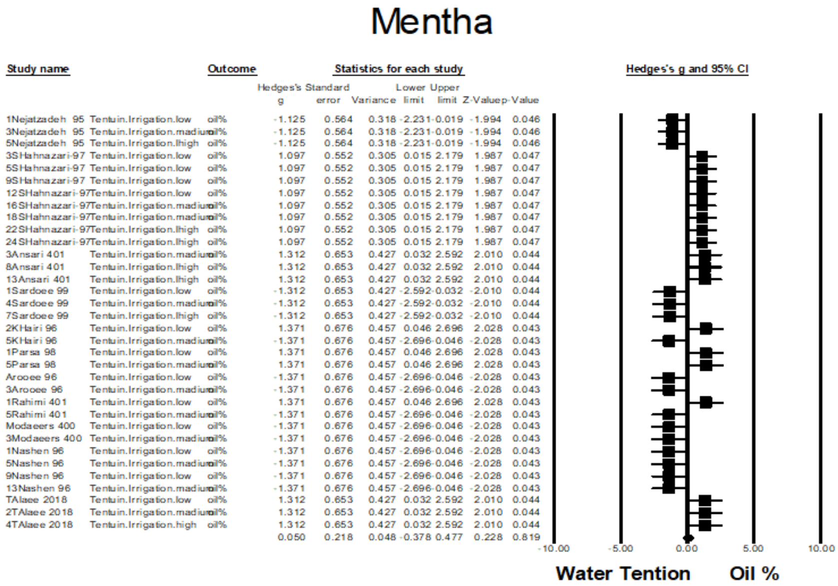

Figure 3- The Forest plot of the effect of water stress (tention) on the essential oil % of peppermint

Table 5 shows the results of the Bag and Mazumdar heterogeneity test. The results show that the studies on the effect of density on the studied traits are all heterogeneous, except for the studies of density on dry weight, which are asymmetric and show diffusion bias. Other studies have no diffusion bias and are symmetrical, and their results can be considered recommendable. The studies of water stress are all heterogeneous and asymmetric, from the study of the stress on the percentage of essential oil, which is symmetric and did not show a diffusion error.

Table 5- Heterogeneity test of the effect of density and water stress on the quantitative and qualitative traits of the peppermint plant with a confidence level of 95%

Traits	I-squared	Z value	P value	Test result
Density- essential oil %	59.600	0.018	0.985	Heterogeneous symmetric
Density-oil yield	42.983	0.994	0.220	Heterogeneous symmetric
Density-height	33.969	1.790	0.073	Heterogeneous symmetric
Density-dry weight	43.272	1.200	0.022	Homogeneous asymmetric
Water tension - essential oil %	85.812	0.474	0.635	Heterogeneous symmetric
water stress- height	0.000	0.769	0.056	Homogeneous asymmetric
Water stress-dry weight	0.000	5.870	0.000	Homogeneous asymmetric
Water stress-oil yield	56.287	4.016	0.000	Homogeneous asymmetric
Water stress-proline	0.000	1.652	0.098	Homogeneous asymmetric

Figure 4 shows the funnel diagram, the study of the effect of density on dry weight, and Figure 5- the diagram of water stress on the percentage of peppermint essential oil percentage. In Figure 4, the asymmetry of density studies on dry weight is seen, and the test results confirm this asymmetry. Figure 5, despite the small number of studies, has relative symmetry and shows no or little diffusion error.

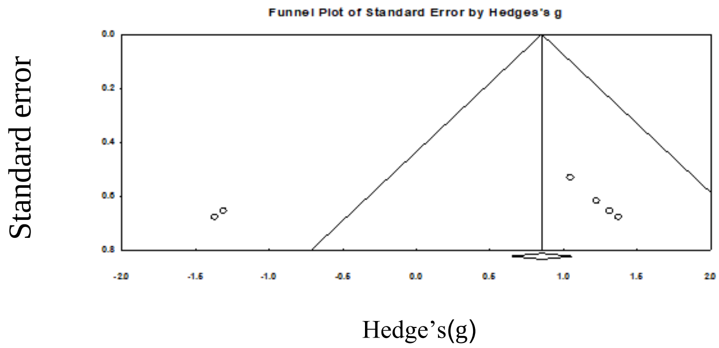

Figure 4 – The Funnel plot of the effect of density on the dry weight of peppermint

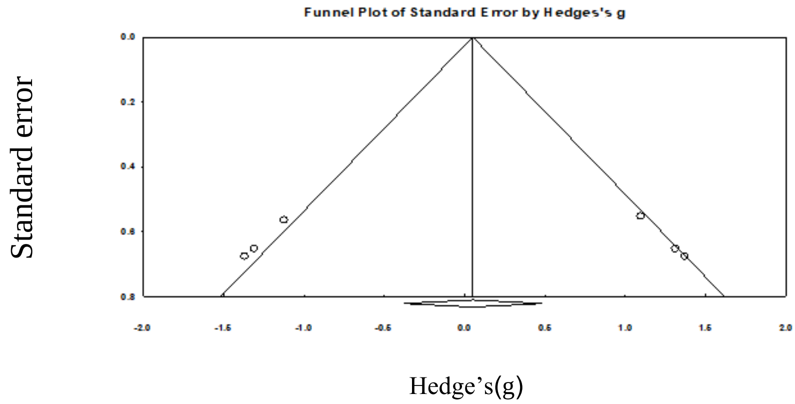

Figure 5- The Funnel plot of the effect of water stress on the peppermint essential oil %

IV. Conclusion

The Meta-analysis of 57 studies on the effect of chemical fertilizer (Nitrogen), water stress, and different planting densities on some quantitative and qualitative traits of peppermint medicinal plants showed that individual studies suffered from calculation errors or errors in the publication of significant printed data. They are wrong. Most of the studies have declared the effect of chemical fertilizer (Nitrogen) on qualitative traits to be significant. In the meta-analysis study, it was observed that the effect of Nitrogen fertilizer on essential oil percentage constituents such as menthol, linalool, and penine and on the amount of proline was not significant. Meta-analysis of the effect of different densities. Planting has been significant on all quantitative and qualitative traits, confirming its significance, and it was observed that the effect on dry weight and height is high and on the percentage of essential oil percentage is moderate. The amount of average effect on the percentage of essential oil percentage has been obtained. The meta-analysis studies of water stress showed a negative and significant effect on quantitative traits, but despite the positive effect on the essential oil percentage, it was not significant. Meta-analysis allows us to examine and calculate its direction and strength with a more detailed and deeper investigation in addition to estimating the amount of impact. It also provides the possibility to guide researchers in the direction of future research by understanding the errors caused by data publication. To reduce errors and increase the accuracy of studies, it is necessary to conduct studies with a larger sample size and repetition in different areas, and researchers can increase the accuracy of the results by calculating the standard error and the size of the effects related to group differences.

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