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Uncovering the Power of Plastic Mulch: Elevating Kohlrabi Yield and Nutritional Value

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Abstract

A leafy vegetable crop commonly grown in Eastern Anatolia, Kohlrabi (Brassica oleracea var. gongylodes) is a major source of nutritional and medicinal compounds. The greenhouse experiment was carried out to assess the influence of plastic mulch (PM) on the morpho-physiological and yield characteristics of kohlrabi. Four kohlrabi cultivars, azur star, kordial F1, lech F1, and kolibri F1, were planted in plastic mulched soil and in uncovered soil. The results showed that plastic mulch film had a statistically significant influence on all cultivars examined. The mulching film increased plant height, bulb weight, and bulb diameter. Kolibri F1 had the greatest plant height and bulb diameter of all four kohlrabi cultivars, with a maximum plant height of 41.7 cm and bulb diameter of 89.02 mm ± 0.83 SE, while azur star had the largest average bulb weight (332.6 g ± 7.2 S.E.) and fiber contents (13.9 mg/g ± 0.75 SE). Kohlrabi cultivars grown with (PM) had lower chlorophyll, total phenolic, and flavonoid contents than those grown without mulch. The highest pH (6.84 ± 0.14 SE) in kolibri F1 gives this sort of an incredible flavor that renders it desirable for consumption. Similarly, in terms of non-enzymatic antioxidant activity, kolibri F1 demonstrated greater percentage inhibition for FRAP and TEAC than other cultivars at $p < 0.05$. The current study indicated that the kohlrabi F1 variety produced excellent results when treated with plastic mulch. Two varieties of kohlrabi (Azur Star and Kolibri F1) were found to be more tasty and more productive when mulching conditions were applied.

Keywords: *Kohlrabi, Organic production system, greenhouse, quality traits antioxidants, nitrate, fiber contents.*

Introduction

Kohlrabi (*Brassica oleracea* var. gongylodes) is cultivated mainly in the winter season and is a biennial vegetable revered for its delicate taste and numerous health benefits (Dhital *et al.,* 2018).

Kohlrabi is extensively grown worldwide, particularly in North America, India, China, Thailand, and northern Vietnam (Choi *et al.,* 2010). Secondary metabolites from plants, a rich source of essential nutrients, are responsible for their bio-active qualities (Lachance and Ramay, 2018). Regular consumption of kohlrabi has proven to minimize the risk of chronic ailments, psychiatric conditions, and various types of cancer (Manchali *et al.,* 2012). Kohlrabi is grown largely for its spherical, thicker stem, which is known as a 'knob' in the world of food. This is caused by a bulging of tissues at the bottom of the stem and, when mature, seems like a turnip. It is frequently eaten as a cooked vegetable. The bulbs, which have a firmness comparable to a radish but a somewhat sweeter flavor, can be cooked, pickled, or eaten raw in salads (Manchali *et al.,* 2012). Cultivars might be white, light green, or purple, and their size and storage capacity vary greatly. Kohlrabi cultivars planted in the spring had bulb diameters of up to 7cm, whilst varieties harvested in the spring can have bulb diameters of up to 20cm and can be preserved for four months under appropriate circumstances (Smychkovich and Hashemi, 2022).

Temperature fluctuations have an impact on knob growth. Early ripening yielded in flowering stalks despite producing knobs, unless temperatures fall down beyond 15ºC for a longer duration. Intense heat results in knob formation with a fibrous and acrid taste (Angmo *et al.,* 2021). Kohlrabi grown in a conventional solar-powered greenhouse in the high-altitude Ladakh mountainous area in the freezing winter produced plants with stunted development, curved leaves, knob splitting, and developing ice crystals due to severe cold stress (Spaldon *et al.,* 2018). The Brassica genus contains a synergistic combination of bio-active molecules that make it a valuable source of food and therapeutic compounds. A wide variety of nutrients are found in its seeds, along with carbohydrates, fats, proteins, and vitamins (Salehi *et al.,* 2021). In addition to glucosinolates, carotenoids and phenolic compounds, there are a wide variety of bio-active metabolites that are important for human health, as well contribute is anti-oxidant, anti-inflammatory, anti-diabetic, anti-proliferative and neuro-protective effects (Salehi *et al.,* 2021; Favela- González *et al.,* 2020).

Mulching is a frequent agronomic and agricultural procedure in which either organic or synthetic material is spread on the top layer of the ground in order to protect it. In addition, organic and artificial (synthetic) mulch is applied to plants (USDA, 1998). In the late 1950s and early 1960s, the United States initiated an attempt to boost commercial output by topping synthetic material (plastic mulch) on a wide range of crops such as brassica, cucumber, brinjal, melon, okra, pepper, tomato, and watermelon. Plastic mulches are part of a plasticulture infrastructure for vegetable production that may also include drip irrigation, fogging, and fertigation (Orzolek, 2017).

Mulching has a number of advantages, including increased soil temperature, lower transpiration rate, regular soil moisture accessibility, weed control, decreased nutrient leaching, and reduced soil compaction, all of which result in higher crop efficiency regarding faster maturity and harvesting (Amare and Desta, 2021; Haapala *et al.,* 2015). On the other side, the

negative repercussions of mulching coatings necessitated higher production expenses for workers in the maintenance and elimination of mulch, which became an important contributor to leftovers (Haapala *et al.,* 2015). The goal of this study was to (A) evaluate the impact of synthetic/plastic mulch on morphological and physiological attributes of Kohlrabi plants in green house and (B) to determine the anti-oxidant potential of four different varieties of Kohlrabi.

2. Materials and Methods

2.1 Experiment Location, Collection and Preparation of the Seeds and Soil

This study was performed in the greenhouse of Nigde Omer Halisdemir University, Turkey. It is lies in latitude 37.97N and longitude 34.68E. It covers an area of 6 km^2 at 1243 meters above the mean sea level. Seeds of four different cultivars (Lech F1, Kordial F1, Kolibri F1 and Azur Star) were obtained from company High Mowing Organic Seed located in USA. Seeds were certified organic by USDA. Seeds were surface sterilized with 0.5% sodium hypochlorite and then thoroughly washed them with sterilized water. Seeds treatment was done to minimize bacterial and fungal infections. Seedlings were sown in plastic seed trays. They were permitted to grow for six weeks until they reached the vegetative stage. The varieties of plants were then relocated to the soil in a greenhouse, both with and without mulch. The soil was prepared for the transplantation of kohlrabi in the greenhouse. Agro-textile material was applied to blocks 1 and 4 of the terrain, while blocks 2 and 3 were left untreated. We created three-row plots in each block/terrain, with the center row being the testing row and the other two being the protective row. The cultivars in each block had three replicas each. Each row contained eight plants with 20 cm spacing between rows and 40 cm spacing between transplants. Mechanical plowing was used to eliminate weeds.

2.1.1 Fertilizer Application and Management Practice

Bio Farm Company supplied organic manure, which was applied at a rate of 170 kg N/ha. Using hand-held equipment, an appropriate amount of fertilizer was sprayed.

2.2 Determination of Germination Parameter and Morpho-physiological Attributes

Ten seeds of four distinct kohlrabi varieties were cultivated in a plastic tray to evaluate the rate of germination. To accurately estimate the percentage of germination, three distinct plastic trays were used and the experiment was carried out three times. At 60-75 days after transplantation (DAT), the plant height was measured with measuring scale and stem/bulb diameter and circumference with a digital caliper. The leaves were cut closer to the bulb and counted for three replicates of each kohlrabi variety in order to estimate the average number of leaves per plant. The weight of the bulb was calculated using an analytical scale. Before weighing the bulb, the leaves were pulled from the bulb's base, and the bulb was cleaned with tap water to remove dirt and soil particles. Furthermore, each kohlrabi variety was spread on white paper or a clean surface to be photographed with a digital camera for evidence. Similarly, the physiological parameters included, total chlorophyll content (TCC), total flavonoid content (TFC), total phenolic content (TPC), pH of kohlrabi juice and total fiber content (TFiC).

2.2.1 Estimation of Chlorophyll Contents

The chlorophyll content was determined using Amon's technique, with minor modifications (Cataldo *et al.,* 1974). Leaf material were considered carefully and homogenized in 80% chilled acetone. The resultant solution was filtrated twice, and the absorbance was taken on a spectrometer at three distinct wave-lengths, notably 633, 645, and 663 nm. Chlorophyll a, chlorophyll b and total chlorophyll was calculated with the help of following formula,

Chlorophyll a = 12.7 x A663 - 2.7 x A645

Chlorophyll b = 22.9 x A645 - 4.7 x A633

Total Chlorophyll = 22.9 x A645 - 4.7 x A633

2.2.2 Estimation of Total Phenolic Contents (TPC)

Total phenolic contents were measured using the methodology adopted by Ozgen *et al,* (2014) with little modifications. 0.5 gram of tested samples were incubated in a buffer comprising acetone, acetic acid, and water for 24 hours in the dark at 4°C. After then, added 0.5 ml of sodium carbonate and 0.5 ml of Folin-Ciocalteus reagents. Then the incubation was done for 40 minutes. In the reaction mixture, 4.5 mL of distilled water were introduced. The absorbance was taken using a UV-visible spectrophotometer at 750 nm.

$$\text{Total Phenolic Content } (\mu g/ml) = \frac{\text{Abs. reading (A) x K value x Dilution factor}}{\text{Weight of fresh tissue}}$$

2.2.3 Estimation of Total Flavonoid Contents (TFC)

Stock solutions of plants in methanol were prepared at a ratio of 4 mg/mL, and were re-suspended in methanol to produce solutions of various concentrations (0.25 mg/mL, 0.5 mg/mL, 0.75 mg/mL, and 1 mg/mL). The test tube was filled with 0.3 mL of 5% NaNO2, followed by 0.3 mL of 10% AlCl3. 5 minutes later, 2 mL of 1M sodium hydroxide were added to the solution. The solution was instantly diluted with 4.4 mL of distilled water. The absorbance of the samples was measured at 510 nm using a spectrophotometer (Phuyal *et al.,* 2020).

$$\text{Total Phenolic Content } (\mu g/ml) = \frac{\text{Abs. reading (A) x K value x Dilution factor}}{\text{Weight of fresh tissue}}$$

2.2.4 Analysis of Crude Fiber

Fiber (crude fiber) was determined by the AOAC approved procedure Filter bag technique with acid cleansing fiber in feed. 0.25ml chemical reagents was added in 100 ml of distilled water and concentrated with addition of few drops of conc. H_2SO_4. Gently heat the solution mixture before adding 2 ml of NaOH solution. The powdered dried samples were prepared. Bags were marked for convenience of identification with one empty bag named Control (C1). Weight and noted the reading of empty bags by digital scale (W1). The prepared samples 0.95-1.00 g were weight in the bags (W2) and checked for correction factor. The bags were sealed with impulse heater and put in the fiber analyzer containing samples. Samples

radially transferred to desiccant pouch to avoid any kind of air and moisture. Then putted in muffle furnace with already measured crucible vessel named and tagged according to the bag for 2 hours at 600 degrees Celsius. Bag/sample in already weighted vessels and cooled conventional dissector and recorded weight loss as W3 (Su *et al.,* 2010).

Calculated % fiber by Formula

% Crude Fiber = 100 * (W3 - (W1 * C1) / W2

2.3 Estimation of Nitrate Level

The nitrate analysis was measure using the procedure of Cataldo *et al*. (1974). The nitrate content of kohlrabi (bulb) powdered was determined. The flask was filled with 20 ml of purified water and 0.2 g of fine bulb powdered. The produced samples were allowed to incubate for one hour at 45 °C until mixed with 0.8 ml of salicylic acid solution and 2-3 drops of concentrated H_2SO_4. Meanwhile, add 19 ml of NaOH to the solution combination and measure absorbance at 410 nm with a spectrophotometer.

2.4 Total Antioxidant Activity (TAC)

The FRAP assay offers a straightforward, rapid, and cost-effective method that eliminate the need for specialize equipment. It was originally employed to measure the reducing power of plasma, but its use has been extended for assessing antioxidant activity in other biological fluids, foods, and plants extracts. The TAEC assay can be used to assess the quality, potency of food and plant extracts. It helps determine the overall antioxidant potential of sample, indicating the effectiveness in combating oxidative stress. In the current research study two different non-enzymatic antioxidant activities i.e. (FRAP and TAEC) was performed for kohlrabi extract to evaluate the antioxidant capacity of plant.

2.4.1 FRAP Activity

Prepare a FRAP reagent using a previously described method by Sutharut and Sudarat, (2012). In brief, it involves the mixing of 300 mM of acetate buffer (pH 3.6), 10 mM of TPTZ solution (2,4,6-tripyridyl-s-triazine) in 40 mM of HCl, and 20 mM of ferric chloride solution in a ratio of 10:1:1. The solution mixture was vigorously mixed. Then take 150 µL of the FRAP reagent and combine it with 20 µL of the sample. Ascorbic acid (20 µL) was used as positive control, whereas distilled water (20 µL) as a blank control. Measure the absorbance of each sample after thirty minutes at wavelength of 593 nm using spectrophotometer.

Percentage of Inhibition = (Control OD - Sample OD/Control OD) * 100

2.4.2 TAEC Activity

To evaluate the antioxidant potential, Ozgen et al., (2014) employed the Trolox Equivalent Antioxidant Capacity (TEAC) technique. Firstly, a methanolic extract was prepared by crushing 6.0 g of kohlrabi bulb and mixing it with 15 mL of methanol. Subsequently, 0.1 mL of the tested sample was mixed with 9 mL of HCl. To this mixture, a few drops of potassium bi-phosphate and 2,2-Aniso-bis, 3-ethylbenzothiazoline (ABTS) was added. The resulting solution mixture was then incubated in darkness for a duration of 12 hours. After the incubation period, 1

mL of the sample and 2 mL of a prepared buffer were combined. The absorbance of this mixture was measured at a wavelength of 734 nm after 10 minutes.

Percentage Inhibition = (Abs. of Control $_{(734)}$ - Abs. of sample $_{(734)}$ / Abs. of Control $_{(734)}$)) * 100

Statistical Analysis

In the current study, the data were analyzed by using SPSS, GPP (Graph pad prism), and Minitab statistics (19.1). The experiment was carried out in triplicate. The mean value plus the standard error (S.E) of every parameter in the study inquiry was determined using one-way ANOVA and further using Fisher LSD for least significance difference between mean value were determined at P value < 0.05.

Result and Discussions

In this study, a quantitative analysis of the yield parameters of four cultivars of kohlrabi using plastic mulch (+) or without mulch (-) was performed. In comparison to plants without mulch, plant covers with mulch significantly boosted seedling germination percentages. Kordial F1 and azur star germinated at 100% when covered with plastic mulch. A 90 % germination percentage was observed in each of the other two cultivars, kolibri F1 and lech F1. The germination rate of all kohlrabi cultivars was significantly lower with plant covers omitted from the soil without mulch. The maximum percentage of germinated seeds for azur star F1 was 80%, whereas the lowest was 70% for the other three cultivars with p = 0.078 as shown in (Figure 1A). Gunawardhana *et al.*, (2011) discovered that soil moisture as well as temperature are important determinants in seed germination. According to their outcomes, seedlings without mulch have little protection against lowering soil moisture caused by high temperatures. Another investigation conducted by Jamil *et al.,* (2007) revealed that when salinity levels increased, kohlrabi percentage of germination and capacity dropped dramatically ($p< 0.001$).

In the present research, plant height shown better growth and development in mulch condition as compared to non-mulch. A significant difference was found between plastic mulch and without mulch regarding plant height at the last harvest. In the analysis of the data, mulching had a significant effect on plant height (F = 11.06 and p = 001). It is shown in figure (1B) that kolibri F1 (41.7 cm) is the tallest plant, followed by lech F1 (39 cm) and azur star (37.2 cm), while kordial F1 (32 cm) is the smallest plant. Conversely, azur star plant covers had the highest maximum height (35.6 cm) and kordial F1 had the lowest (27.5 cm). An earlier study by Shinde *et al.*, (1999) showed differently color plastic mulches had contradictory effects on vegetables like chillies, broccoli, brinjal, and tomatoes. Their findings supported the present experimental work by showing that plastic mulch application retains soil moisture and optimum soil temperatures better than control, which improved plant growth.

The statistical analysis also revealed a significant increase in bulb diameter in kohlrabi cultivars under mulch treatment compared to the control, with p = 0.019 less than $p< 0.05$. Among four different kohlrabi cultivars, the average bulb weight ranged between 281.8–332.6 grams when treated with plastic mulch, compared to 257–305.76 grams without mulch. At $p< 0.05$, azur star had the highest average bulb weight (332.6 g ± 7.2 S.E.) and kolibri F1 had the lowest (281.8 g ± 5.6 S.E.). In comparison, control plants had a substantially lower average

weight of bulb among kohlrabi, which was identified as azur star 305.75 ± 10.75 S.E, lech F1 312 ± 15 S.E, kordial F1 290 ± 13.5 S.E, and kolibri F1 257.2 ± 5.1 S.E (Figure 1D). The ripening stages and four different varieties of kohlrabi at time of harvest were shown in Figure 2 and 3, respectively. According to fewer researchers, plastic mulching raises soil temperatures by trapping solar radiation, reduces loss of moisture to the soil and improves water-use efficiency, generates more photo assimilates, and raises source and sink size during crop growth, all of which lead to higher crop production. Prior study of Ahmed *et al.,* (2003) concluded that combine application of plastic mulch and fertilizer (NPK) increased plant yield efficacy in kohlrabi. In the same way, another research of He *et al.,* (2017) reported that plastic mulch applications interestingly increase yield output in cereal crops. Our result shown increase in bulb weight by mulch treatment were supported by outcomes of earlier studies.

In addition, average bulb diameter in four distinct kohlrabi cultivars were statistically analyzed keeping level of significance at 0.05. It was observed that, bulb diameter in kohlrabi had shown marginal variation in both plastic mulch and control treatment. Kordial F1 cultivar has the largest average bulb diameter was 89.02 mm ± 0.83 SE, followed by Azur star and Kolibri F1 cultivars (88.26 ± 2.88 SE, and 86.18 ± 0.74 SE). The Lech F1 cultivar had the least average bulb diameter of 85.39 mm ± 3.35 SE as shown in figure 1C. Whereas, maximal (79.9 ± 3.6 SE) average bulb diameter was found in kordial F1 and lowest 78 mm ± 3.67 SE, in lech F1. Moreover, the average bulb height of four different cultivars of kohlrabi was varied from 61.88 mm to 68.99 mm in plastic mulch treatment, while without mulch ranged between 57.80 mm - 71.77 mm. Their found non-significant difference between both treatments. Our results are similarly consistent with those reported by (Stephen *et al.,* 2006), they found average bulb diameter of 100.2 - 62.7 mm. The increase in bulb diameter and weight of bulb directly correlates and depends on water use efficiency during plant development. Similarly, the present study was compared to Zutic *et al,* (2016), which concluded that mulching has shown positive effect on fruiting/yield parameters.

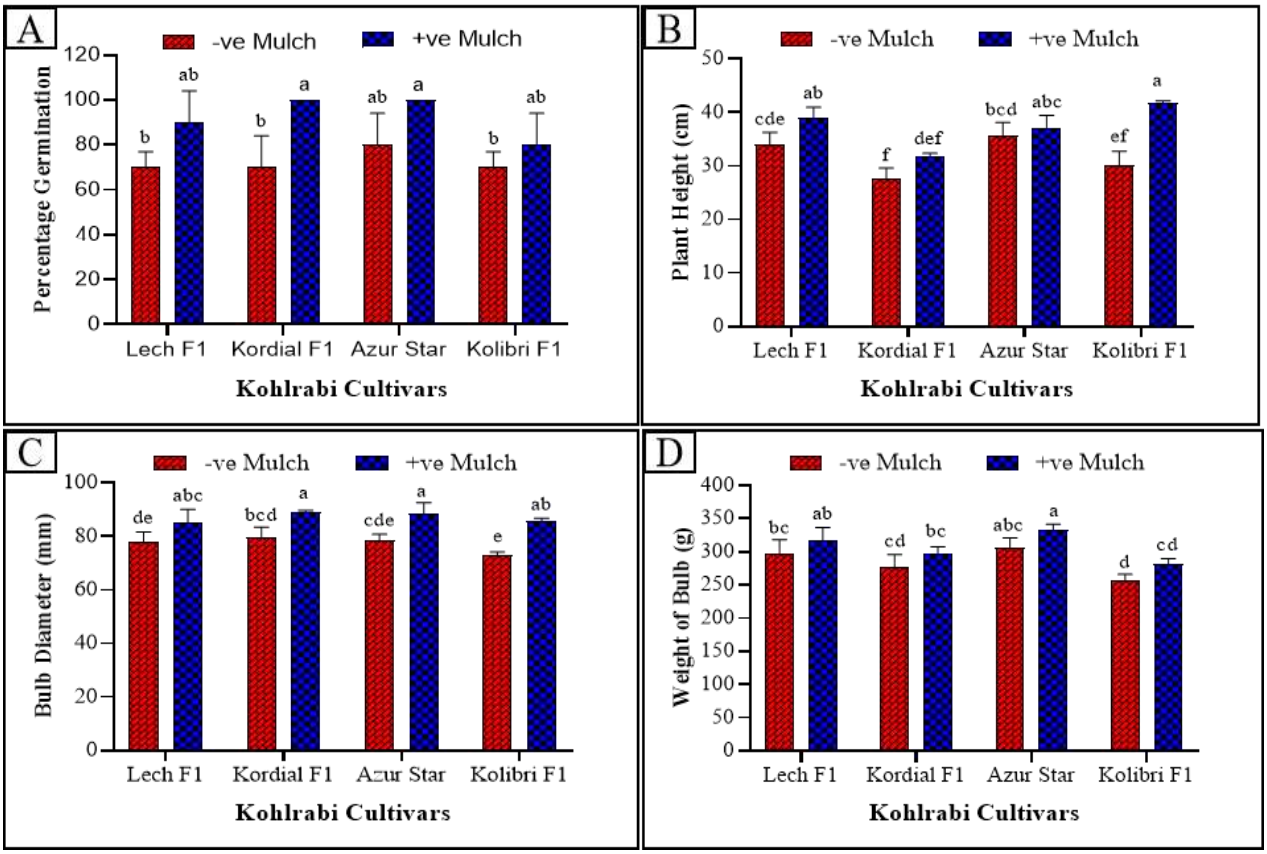

Figure 1: Effect of treatments of plastic mulch and without mulch on morphological attributes of kohlrabi. A) Percentage germination, B) Plant height, C) Bulb diameter and D) Weight of bulb in gram for four different kohlrabi cultivars. Bars sharing similar letters do not show a significant difference ($P< 0.05$) as computed by ANOVA followed by Fisher LSD test using software, Minitab 19. Vertical bars show standard error (SE) of means of three replicates.

Seedling stage	Vegetative stage	Fruiting stage

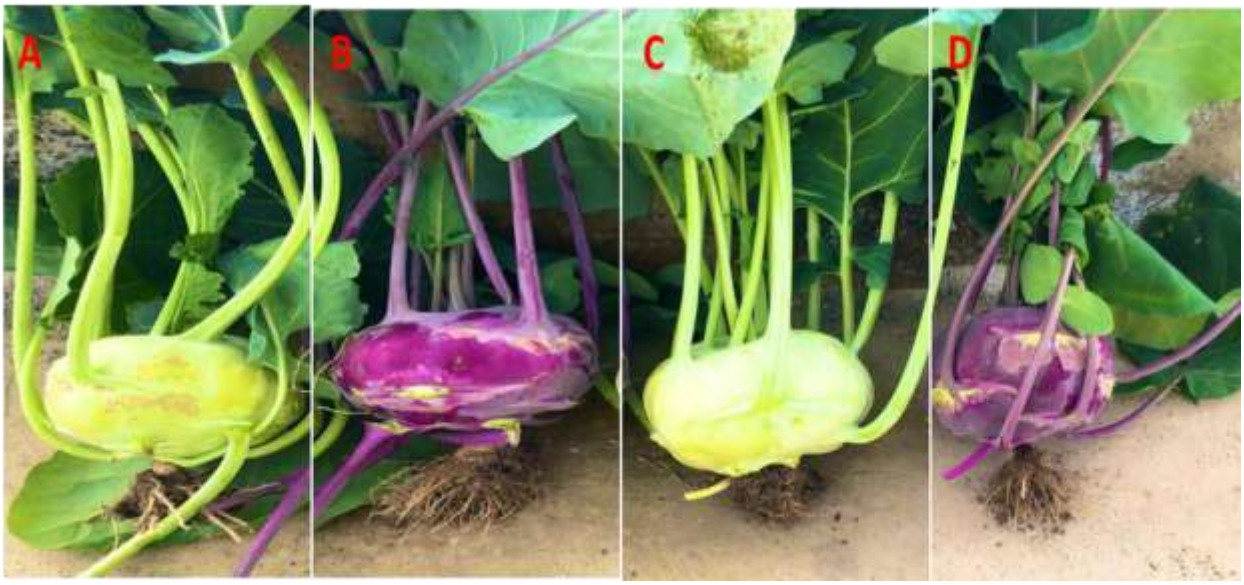

Figure 3: Four different cultivars of kohlrabi, (A)Lech F1, (B) Azur Star, (C) Kordial F1, (D) Kolibri F1.

Physiological Parameters

The process of photosynthesis plays a significant role in plant development and contributes largely to the increase in yields. Variations in the soil microclimate are generally thought to affect fluctuations in photosynthetic activity. The statistical analysis of the data showed that the levels of chlorophyll in the control condition were greater than that in the plastic mulch (PM) at P 0.005. The overall average chlorophyll concentrations in kordial F1 were 31.34 mg/g ± 0.50 SE, subsequently followed by kolibri F1 at 31.06 mg/g ± 0.7 SE and lech F1 at 30.2 mg/g ± 1.13 SE. Furthermore, the azur star with the condition without mulch exhibited the lowest mean chlorophyll of 26.28 mg/g ± 0.5 SE. In contrast, kohlrabi cultivars cover with mulch, with lech F1 having the highest average chlorophyll content of 24.76 mg/g ± 0.26 SE and kordial F1 having the least amount of chlorophyll at 23.4 mg/g ± 0.84 SE (Figure 4A). Helaly *et al.,* (2017) and (Eissa, 2002) suggested that a rise of chlorophyll contributed to higher nitrogen absorption and a rise in soil microbial population owing to applying mulch, which produced exactly the opposite results as the current findings. The current study hypothesized that chlorophyll is an essential component of the light reaction of photosynthesis since chlorophyll b is a light-harvesting pigment that captures extra energy, which is subsequently transferred onto chlorophyll a. So, based on our findings, kohlrabi cultivars grown without mulch showed an increase in chlorophyll, which might be attributed to direct sunlight exposure. A plant's photosynthesis contributes to its growth and development, which is directly related to its yield performance (Zou *et al.,* 2006).

In the current study, statistical analysis revealed that both plastic mulch (PM) and no mulch treatments had a non-significant rise in the pH value of bulb juice ($F= 3.67$, $p = 0.044$). It was discovered that the Kohlrabi bulb at a lower pH level is more acidic than those with pH values closer to pH 7. It was also discovered that cultivars without mulch treatment had higher

pH (6.84 ± 0.14 SE) in kolibri F1 and lower (6.31 ± 0.25 SE) in kordial F1, whereas plastic mulch had higher acidic pH (6.82 ± 0.22 SE) in kolibri F1 and lowest (6.18 ± 0.28 SE) in kordial F1 among all four kohlrabi cultivars (Figure 4B). The pH is an essential parameter strongly associated to food characteristics such as texture, flavor and aroma. Alkalinity and acidity (pH) of food both have a life-threatening impact on the body therefore body tries to maintain with in the threshold range 6.5-7.5. According to statistics, consuming alkaline foods can help prevent osteoporosis (Cunninghum, 2009). Shams, (2012) findings showed that both mulch and bio-fertilizer treatments have a beneficial influence on kohlrabi yield evaluations, but contrasted with our findings. Their investigation concluded a higher pH (acidity), yet the research we conducted found a lower value. Similarly, Alagoz *et al.,* (2020) found that fruit shape indexes, color, and pH-levels did not differ significantly between mulch treatment and control groups.

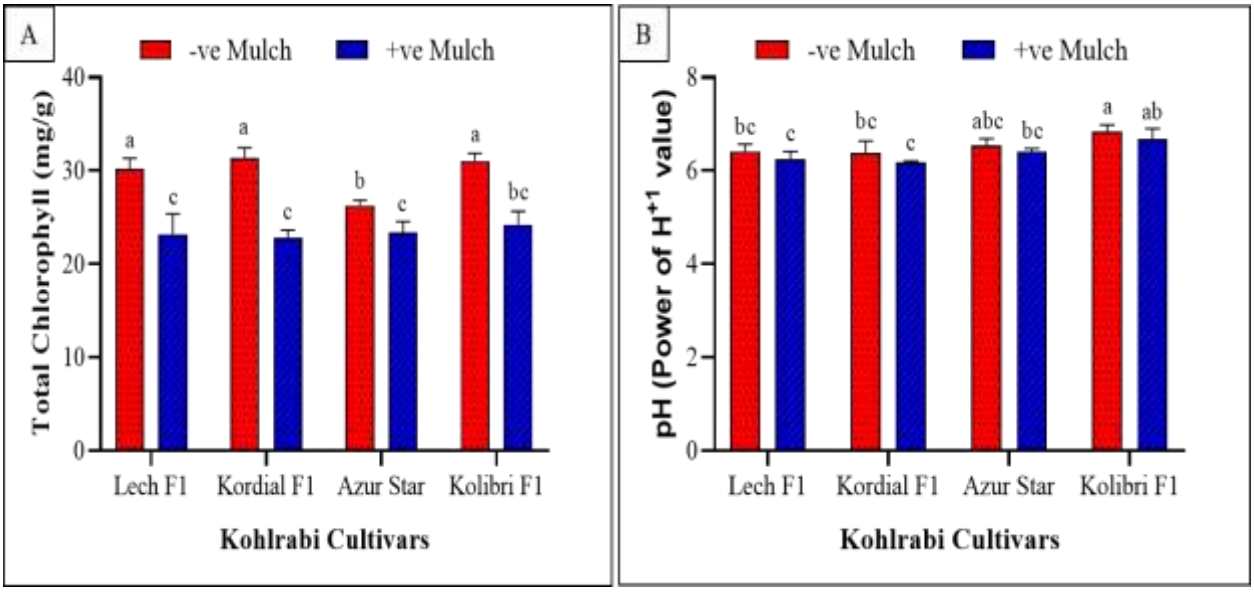

Figure 4: A) Effect on both plastic mulch and without mulch treatment on chlorophyll contents in chilling stress condition. B) Effect of plastic mulching and control on pH of bulb juice under chilling stress condition. Bars sharing similar letters do not show a significant difference (P< 0.05) as computed by ANOVA followed by LSD test using software, Minitab 19. Vertical bars show standard error (SE) of means of three replicates.

In addition, the fiber content in four different cultivars of kohlrabi varied statistically non-significant between the plastic mulch and the control group under chilling stress. The p-value of 0.342 with F=1.34 revealed that the fiber content values were statistically insignificant. The analyzed results, meanwhile revealed higher fiber contents (13.9 mg/g ± 0.75 SE) in azur star under plastic mulch treatment, but the results are inconsistent across both treatments. As both plastic mulch and no mulch (control) were examined. Kordial F1 had the lowest fiber content (12.43 mg/g ± 0.43 SE) as shown in (Figure 5A). The outcomes of Divisi *et al.,* (2006) reported 13.1% increase in crude fiber contents, that was approximately similar to that of our study.

Similarly, the effect of plastic and without mulching on four distinct kohlrabi cultivars were tested to see whether there were any differences in crop physiology under cold stress conditions. In the current study, it was shown that plants without mulch treatment had greater phenolic levels than those with mulch. Secondary metabolites, which are usually found within plants, and phenolic and flavonoid compounds are highly effective antioxidants that prevent plants from stress (Tungmunnithum *et al.,* 2018). According to our findings, kolibri F1 has the highest 44.6 mg/g ± 1.4 SE, preceded by lech F1 (41.6 mg/g ± 2.0 SE) and kordial F1 (37.3 mg/g ± 0.5 SE). The azur star showed the least phenolic content (34.1 mg/g ± 0.6 SE) because no mulch had been applied during chilling stress (Figure 5B).

Plant coverings with plastic mulch, on the other hand, reduced overall phenolic content. Kolibri F1 had the greatest value (36.7 mg/g ± 1.5 SE), while Kordial F1 had the lowest value of 28.2 mg/g ± 1.8 SE. According to statistical analysis, the p-value for phenolic content was significant at p=0.001 and F=18.4, which was close to the level of significance (p=0.05), demonstrating that the phenolic content results are highly significant. Previously, Sharma *et al.,* (2008) conducted research on a strawberry and found that the application of plastic mulching demonstrated fluctuation in phenolic levels in three distinct developmental phases of plant growth, however, those findings were contradictory to the present study. We discovered that plants with no mulch generated additional phenolic compounds compared to plants with plastic mulch; nevertheless, we were more confident in the current finding since plants subjected directly to freezing stress produced more phenolic for plant survival. Similar study was performed by Elmastas *et al.,* (2017) observed that, flavonoid and phenolic level fluctuated in plant vary to various developmental stages and also concerns with climatic condition.

The total flavonoid contents among kohlrabi cultivars under cold stress condition have been examined statistically at p = 0.05, and the results for flavonoid were discovered that p-value (p=0.000, and F= 36.05) less than the level of significance (p = 0.05) is highly significant. Surprisingly, the plants that did not have plastic mulch coverings accumulated more flavonoids than those that did. Kolibri F1 has a greater flavonoid content of 39.2 mg/g, whereas azur star as plant coverings without mulch has the lowest flavonoid level of 22.2 mg/g (Figure 5C). Further, kohlrabi varieties covered with plastic mulch accumulated less flavonoid accumulation; additionally, kolibri F1 had greater 28.3 mg/g flavonoid levels and least 16.2 mg/g for lech F1 under cold stress conditions (Figure 5C). Prior study conducted by Saeed *et al.,* (2019) on various fruits and vegetables varieties, and has shown inconsistent results to both mulch and control group, which was dissimilar to our outcomes in which we found more flavonoid accumulation in without mulch treatment in chilling stress.

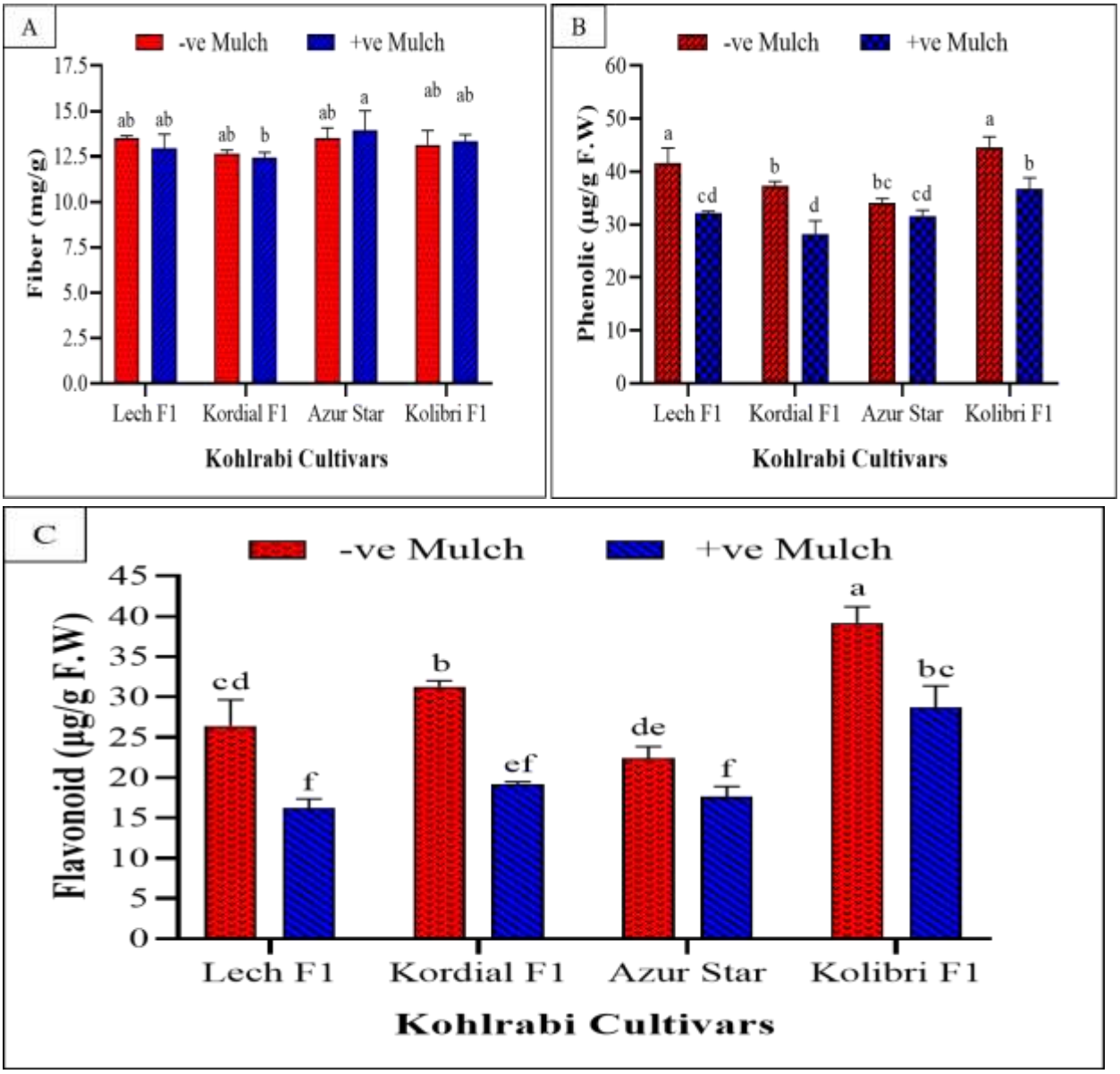

Figure 5: Effect of treatment of plastic mulch (+ve) and without mulch (-ve) on plant physiology. A) Fiber contents, B) Phenolic contents, C) Flavonoid contents among four different kohlrabi cultivars. Bars sharing similar letters do not show a significant difference ($P< 0.05$) as computed by ANOVA followed by Fisher LSD test using software, Minitab 19. Vertical bars show standard error (SE) of means of three replicates.

Nitrate Analysis

In the present research, mulching was carried to assist insulation against extreme low temperature as compared to control (-ve mulch). It was found that plastic mulching had a

moderate increase in nitrate contents. In extremely cold conditions, nitrate level range from 2.25 g/kg to 3.74 g/kg DW in treatment with and without mulch. During extreme cold, a statistically significant p-value ($p< 0.05$) was found for nitrate concentrations within the plastic mulch and control. When kohlrabi was encapsulated with mulch, the most significant nitrate content was 3.74 g/kg DW ± 0.09 SE in lech F1 and its lowest value was 3.11 g/kg DW ± 0.01 SE in azur star (Figure 6). When the data was quantitatively examined, $p=0.001$ confirmed that the results are significant for nitrate contents. A maximum of 3.34 g/kg DW ± 0.09 and a minimum of 2.25 g/kg DW ± 0.05 SE occurred in kolibri F1 plants that were immediately exposed to cold conditions (Figure 6). The current study was compared to previous findings of Santamaria *et al.,* (1999), who reported that nitrate concentrations readily accessible to plants varied according to seasonal crops, whereas in the current study, plastic mulch traps more heat from sunlight, which leads to arise in nitrates absorption in comparison to the control. Soil-retained moisture and microbial communities have promoted nitrogen buildup, and plants acquire nitrogen from soil mostly in nitrate form. Increased nitrogen uptake leads to improved plant growth and yield performance.

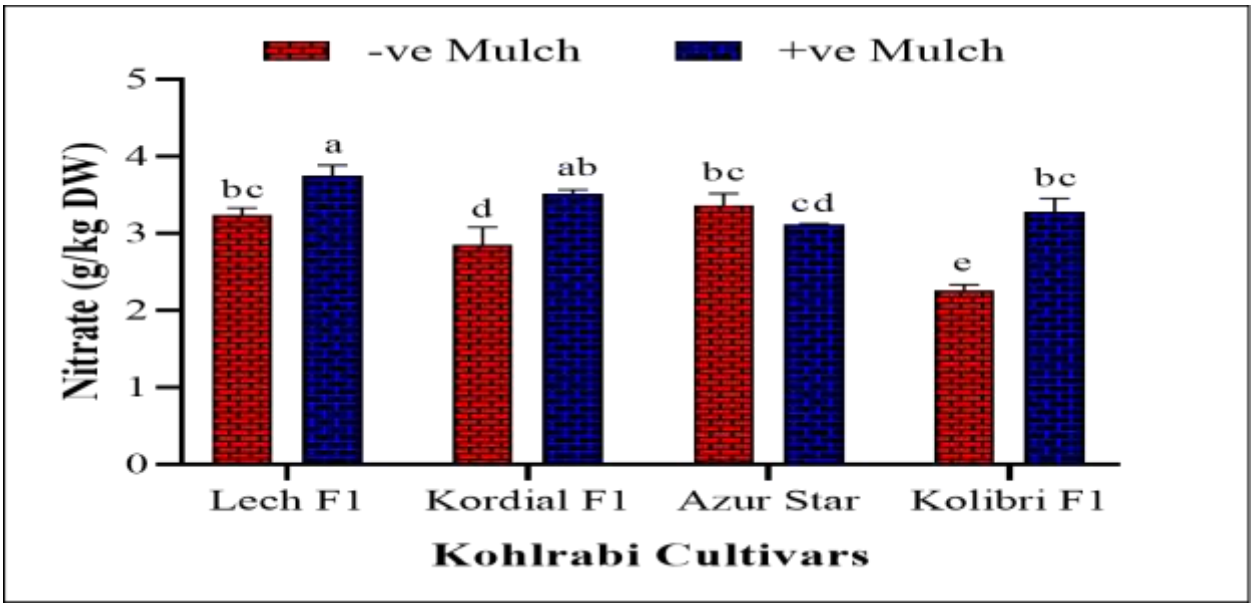

Figure 6: Effect of plastic and without mulch on nitrate contents among different kohlrabi cultivars. Bars sharing similar letters do not show a significant difference ($P< 0.05$) as computed by ANOVA followed by Fisher LSD test using software, Minitab 19. Vertical bars show standard error (SE) of means of three replicates.

Antioxidant Activities

In an oxidation reaction, free radicals such as hydrogen peroxide, superoxide anion, and hydroxyl ions are produced. If these free radicals occur in a cell, they can trigger chain reactions that can cause obliteration or even death. A chemical reaction known as oxidation occurs when hydrogen or electrons are transferred from one material to another. Antioxidant molecules within

the cell prevented molecular oxidation. Antioxidants inhibit other oxidative processes by eliminating intermediates of free radicals and preventing the chain reaction from continuing. The data analyzed for antioxidant activity showed significant differences among four kohlrabi cultivars when plastic mulch and control were compared under chilling stress.

In the case of FRAP activity (Table 1), the p-value was equal to ($p = 0.034$ and $F = 4.03$). There was a greater percentage of inhibition on the mulch-covered plants than on the control plants. As a result of cold stress, kolibri F1 showed a maximum of 81% inhibition, followed by kordial F1 at 78% and lech F1 at 74%, whereas azur star showed a minimum of 67% inhibition (Figure 7A). Conversely, plants without mulch showed higher FRAP inhibition for kolibri F1 while azur star had the lowest inhibition (75% and 58%). The decrease in antioxidant activity caused by the usage of polyethylene mulching is consistent with the trend reported in tomato research by Valková *et al.,* (2018). Marti *et al.,* (2011) conducted an identical investigation on several cultivars of various vegetables and found significant variations in total antioxidant effectiveness were varied due to environmental factors. According to current study findings, the percentage inhibition of various kohlrabi cultivars under cold stress conditions showed diverse responses.

Antioxidant plays a vital role against free radical, which play an important role of antioxidant as played in human body as source of immunity against debilitation and chronic diseases. Another activity of non-enzymatic antioxidant assay (TEAC) was done to check scavenging potential of four different varieties of Kohlrabi cultivars. When the TEAC antioxidant assay was checked out both with and without plastic mulch treatments, a statistically significant difference of $p< 0.05$ was detected. Data analysis examined the TEAC antioxidant assay findings, which were statistically significant with $p = 0.008$ (Table 1). When cultivars were infused with plastic mulch, the highest 86% and lowest 70% inhibition percentages were recorded for kolibri F1 and azur star, respectively (Figure 7B). Plants were grown lacking mulch, on the other hand, showed a somewhat lower inhibition percentages for all four kohlrabi varieties. It was discovered that azur star had the greatest percentage inhibition of 68%, followed by kordial F1 (66%) and kolibri F1 (64%), with leach F1 exhibiting the lowest recorded value of 59% (Figure 7B). The present study outcomes were compared to Benajiba and Khojiah, (2021) shown opposites results to each other. This discrepancy in both findings might be attributed to a wide range of climate conditions that produced remarkably contradicting outcomes, along with the adoption of distinct cultivars in both studies.

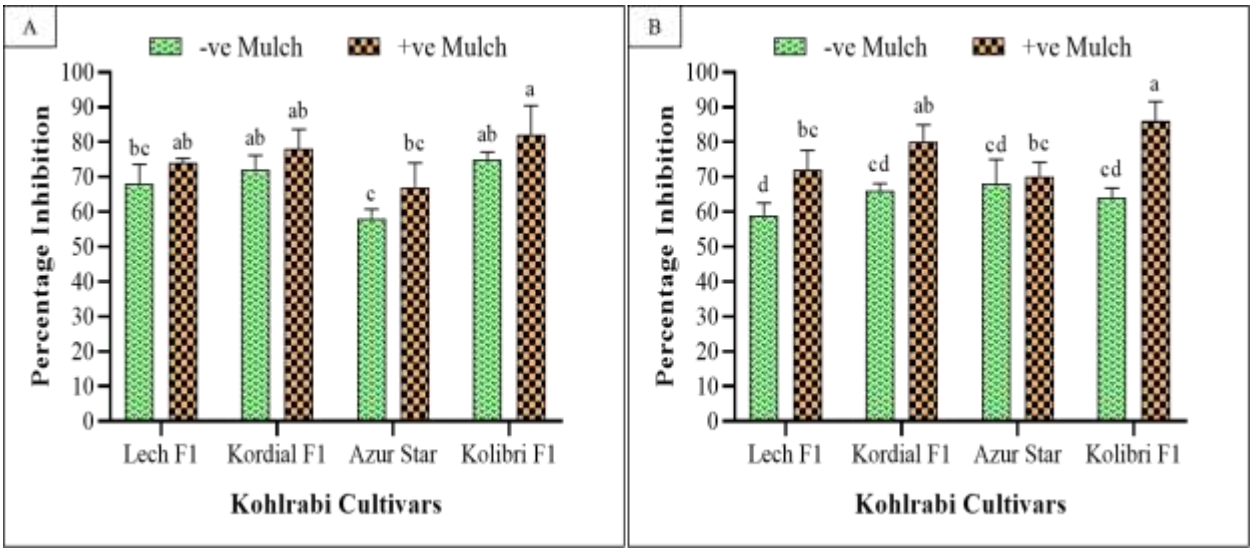

Figure 7: Non-enzymatic assay in four kohlrabi cultivars in both plastic and without mulch treatments. A) Percentage inhibition by FRAP assay in kohlrabi cultivars, B) Percentage inhibition by TEAC assay in kohlrabi cultivars. Bars sharing similar letters do not show a significant difference ($P< 0.05$) as computed by ANOVA followed by Fisher LSD test using software, Minitab 19. Vertical bars show standard error (SE) of means percentages of three replicates.

Table 1: FRAP and TEAC activities with average percentages, standard deviation, standard error (SE), F value and p-value calculated for four kohlrabi cultivars.

Kohlrabi (Cultivars)	Treatment	N	Mean (FRAP)	Mean (TAEC)	St Dev, SE (FRAP)	St Dev, SE (TEAC)	F value, p-value (FRAP)	F value, p-value (TAEC)
1. Lech F1	-ve Mulch	3	68.00	59.00	5.66, 2.02	3.54, 1.78	F = 4.03 P-value = 0.034 which is smaller than p = 0.05	F = 6.72 P-value = 0.008 which is smaller than p = 0.05
2. Kordial F1		3	72.00	66.00	4.64, 2.28	2.12, 1.5		
3. Azur Star		3	58.00	68.00	2.83, 0.64	7.07, 2.44		
4. Kolibri F1		3	75.00	64.00	2.32, 1.7	2.83, 0.64		
5. Lech F1	+ve Mulch	3	74.00	72.00	1.41, 1.01	5.66, 2.02		
6. Kordial F1		3	78.00	80.00	5.66, 2.02	4.95, 2.48		
7. Azur Star		3	67.00	70.00	7.07, 2.44	4.24, 2.22		
8. Kolibri F1		3	81.00	86.00	8.49, 6	5.66, 2.36		

Conclusions

The present results indicate that the use of plastic mulch, as a good for the growth of kohlrabi cultivars is a viable approach. This approach was applied to vegetables for increment of yield and as well as for lowering the soil moisture and retention of temperature for better plant growth and development. The plastic mulch treatment significantly increases plant height, bulb diameter, weight of bulb, total fiber contents and antioxidant activities. However, it results in significantly reduction of total chlorophyll contents, total phenolic and flavonoid contents. Moreover, plastic mulching enhanced the nitrate accumulation for plant from soil by increasing microbial diversity. These results indicate some positive effects of plastic mulch utilization as an alternative substrate for growth of kohlrabi that must be further explored.

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