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African indigenous leafy vegetables**

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1. Introduction

African indigenous leafy vegetables (AIVs) are currently referred to as ‘underutilised species’ (Virchow, 2003). Public awareness of these species has continued to increase since they were first brought into the limelight in 1992 by the Convention on Biological Diversity and the Global Plan of Action for Plant Genetic Resources for Food and Agriculture (Virchow, 2003). Since then, a number of organisations and most recently the German ministry (BMBF/BMZ) funded project Horticultural Innovation and Learning for Improved Nutrition and Livelihood in East Africa (HORTINLEA), under the funding initiative “Securing the Global Food Supply – Globe”. HORTINLEA is focussing on investigating and promoting these crops as a strategy to improve livelihood and nutrition of the rural, peri-urban and urban inhabitants in developing countries, especially Kenya.

AIVs referred to in this case might not be indigenous to the country, but are associated with traditional production systems, local knowledge and usually have a long history of local selection and usage. Their role in improving living standards of resource-limited communities has long been recognised by ethno-botanists who have even documented traditional knowledge associated with these species (Maundu et al., 1999). In the past, AIVs (e.g. African nightshade (*Solanum* spp.), vegetable amaranth (*Amaranthus* spp.), spiderplant (*Cleome gynandra* L.), cowpea (*Vigna unguiculata* (L.) Walp), Ethiopian kale (*Brassica carinata* L.), were to be found only in the backstreets and in a few open-air markets. Today, marketing and consumption of these vegetables, in Kenya have changed. Currently, they are competitively sold in most supermarkets and municipal markets of major towns and cities, in increasing quantities on a daily basis alongside the most commonly consumed exotic (introduced) vegetables. These exotic vegetables include cabbage (*Brassica oleracea* L. var. *capitata* f. *alba*), chard (*Beta vulgaris* subsp. *cicla* (L.) W.D.J. Koch); commonly known as spinach in Kenya and collard greens (*Brassica oleracea* L. var. *viridis*); known as sukumawiki or kale in Kenya (Chelang’a et al., 2013; Onyango and Imungi, 2007). In Kenya, the most consumed AIVs (e.g. African nightshade and vegetable amaranth) serve as useful sources of vitamin A, Fe, and Zn. Malnutrition and the global nutrient problem known as “hidden hunger” are associated with deficiencies in these micro-elements (Gogo et al., 2016). They are also reported to be rich in proteins, fibre and minerals such as Na, P, and Ca (Abukutsa-Onyango, 2003). Recently, attention is being directed to these vegetables because of their high contents of bioactive secondary compounds such as carotenoids, flavonoids, phenolic acids, and other phenolic compounds (Castrillón-Arbeláez and Délano-Frier, 2016; Nana et al., 2012; Noori et al., 2015). These substances possess strong antioxidant and possible anticarcinogenic properties and have recently been implicated in health promotion (Kasote et al., 2015; Nana et al., 2012; Noori et al., 2015; Odongo et al., 2017).

In many cases AIVs are well adapted to the agro-ecological conditions, thrive well under minimum resource conditions, thus have a comparative advantage in marginal lands over exotic vegetables and may contribute to low-input sustainable production systems (Abukutsa-Onyango, 2003).



Overwhelming demand of AIVs has stimulated many entrepreneurs, especially women and youth, to grow and trade these vegetables on a small-scale basis (Chelang'a et al., 2013; Muhanji et al., 2011). Opportunities exist in Kenya to use AIVs to expand the local food base, improve nutrition and health of the population, enhance food security and generate income. Therefore, AIVs fit well in the achievement of the sustainable development goals (SDGs), especially the first three (no poverty, zero hunger and good health and wellbeing). The commonly produced and marketed AIVs include African nightshade (*Solanum* spp.), vegetable amaranth, spiderplant, cowpea, Ethiopian kale, slender leaf/rattle pod (mitoo) (*Crotalaria ochroleuca* G. Don. and *C. brevidens* L.), jute mallow (*Corchorus olitorius* L.), and pumpkin leaves (*Cucurbita maxima* L. and *C. moschata* L.) (Baldermann et al., 2016; Maundu et al., 1999). However, for most of these species, documented scientific knowledge is either rare or only beginning to emerge, especially on postharvest losses, further complicating the intervention process.

The main constraint to increased production, marketing and consumption of AIVs is its high perishability and low storage capacity in fresh form (Onyango and Imungi, 2007). AIVs display high metabolic activity after harvest hence, liable to faster postharvest deterioration in quantity and quality. Currently, the magnitude of postharvest losses of AIVs in Kenya can be as high as 50%, depending on the species (Gogo et al., 2016). These losses are attributed on one hand to pre-harvest factors (e.g. inadequate nutrition, irrigation, crop protection); affecting yield, harvest and postharvest quality and on the other hand to postharvest factors (e.g. inappropriate postharvest handling and treatments); resulting in rapid physiological deterioration, loss of nutritional quality and microbiological decay during the supply chain (Onyango et al., 2009; Onyango and Imungi, 2007). The commonly used postharvest treatment methods include air-drying, solar-drying, blanching, and fermentation (Gogo et al., 2016). However, despite their wide adoption for many years, these methods are reported to result in significant loss of nutritional product quality (Muchoki et al., 2007). In the past few years there has been increasing interest in the rediscovery of traditional postharvest treatments (e.g. sun/solar drying, blanching, fermentation), on one hand and on the other hand, on new emerging pre- and postharvest technologies (e.g. electric current, UV-C irradiation, modified film packaging). Numerous studies have shown the beneficial effects of these postharvest treatments to control insect pests, prevent fungal rots, and inhibit undesired acceleration of ripening and senescence or even to promote the synthesis of health promoting compounds during storage and marketing (Artés-Hernández et al., 2009; Chairat et al., 2013; Dannehl et al., 2011; Huyskens-Keil et al., 2011; Khalili et al., 2017). These easy-to-apply postharvest treatments can prevent quality losses effectively and can substitute chemical preservation with non-damaging physical treatments that meet hygienic requirements; to develop new food safety regulations for AIVs and to prolong their shelf life as well as their storability. In addition, based on the challenge of availability, reliability and cost of electricity these postharvest treatments might be applicable instead of cooling facilities (Yadoo, 2012).

Aim of the present study was to assess the situation of postharvest losses during AIVs supply chain (from smallholder farmer to consumer), determine the amount of postharvest loss (quantitative and



nutritional) along the supply chain in Kenya. Thereafter, a series of studies were conducted on pre-harvest (electric current) and postharvest (UV-C irradiation) treatments to determine their effects on primary compounds (chlorophylls, mineral elements, proteins and dietary fibre) and secondary metabolites (carotenoids, flavonoids, phenolic acids, phenolic compounds, glutathione peroxidase (GPOX), and vitamin E) and microbial status as well as antioxidant capacity, in order to strengthen a product quality and safety oriented food supply chain. The study focussed mainly on two commonly consumed AIVs i.e. Vegetable amaranth (*Amaranthus cruentus* L. cv. Madiira) and African nightshade (*Solanum scabrum* Mill. cv. Olevolosi).



2. Scientific background

2.1. Traditional pre- and postharvest handling, treatments and losses of AIVs in Kenya

Pre- and postharvest treatments of agricultural produce are one of the central problems developing countries are facing resulting in low yield and poor quality AIVs, including Kenya (Table 2.1). Owing to the lack of and/or inadequacy of pre- and postharvest treatment technologies, large quantities of urgently needed food, especially AIVs, are lost (Table 2.2). Major postharvest losses reported in Kenya are due to insufficient pre-harvest conditions, insect pest and diseases, poor storage conditions, and poor handling along the supply chain (Gogo et al., 2016). These unfavourable conditions are even more serious during rainy and dry seasons where the vegetables exist in abundance and scarcity, respectively. During this period when AIVs are scarce, many rural, peri-urban and urban dwellers have a limited availability of leafy vegetables; which contributes to lack of dietary diversity or malnutrition of the local populations. Postharvest handling and treatments (e.g. cleaning, sorting, grading, cold storage, packaging, blanching, drying, and fermentation) in Kenya have been reported to improve shelf life and quality as well as reducing microbial contamination of AIVs (Ayua and Omware, 2013). On the other hand, other studies indicate that drying and blanching may reduce quality of AIVs (Chege et al., 2014; Kasangi et al., 2010). However, farmers in Kenya still rely on traditional postharvest treatment methods; including sprinkling cold water on leaves to maintain freshness, sun-drying, unconventional packaging (gunny bags and non-perforated polythene bags). In Africa and Kenya in particular, the lack of and/or inadequacy of postharvest treatment technologies exists with many vegetable varieties (especially AIVs) resulting in wastage during the in-season (late March to June) and limited supply during the off-season (December to April) accompanied by high prices (Habwe et al., 2008), because most locally available vegetables are seasonal and not available year-long. AIVs cannot be marketed fast enough when they are in-season owing to their high perishability and thus, limited shelf life and storability. This forces farmers to sell soon after harvest (Shiundu and Oniang'o, 2007). Onyango and Imungi (2007) reported 3.1%, 3.5%, 4.2% and 5.5% losses of spider plant, African nightshade, cowpeas and vegetable amaranth, respectively, due to wastage as a result of excessive wilting, in Nairobi groceries alone. Accordingly, market sellers and supermarkets strive to sell all the supplies on the day of delivery and whatever remains at the end of the day may be discarded as having lost saleable value. This was reported to be a major problem of AIVs sold in urban centres contributing to heavy postharvest losses (Onyango and Imungi, 2007). In addition, wilting was also indicated to be a challenge as AIVs deteriorate faster especially at ambient retailing conditions where trading of these vegetables is mostly done. Appropriate pre- and postharvest treatments and adequate storage methods are necessary in order to ensure quality and the availability of these nutrient-rich foods all year round (Habwe et al., 2008).

Table 2.1: Leaf yield and nutritional value (per 100 g fresh edible portion) of selected Kenyan African indigenous leafy vegetables (AIVs).

| Scientific name | Harvesting stage (weeks)* | Yield ton ha ⁻¹ | Crude protein (g) | β-Carotene (mg) | Vit. A (mg) | Vit. C (mg) | Ca (mg) | Fe (mg) | Dry matter (g) |
|-------------------------------------|---------------------------|----------------------------|-------------------|-----------------|-------------|-------------|---------|---------|----------------|
| <i>Vigna unguiculata</i> (L.) Walp. | 4-8 | 5.4-10 | - | 6-8 | 5.7 | 70-100 | 152-400 | 10-15 | 15-20 |
| <i>Solanum</i> spp. | 4-8 | 30-80 | 3-6 | 8-10 | 8.8 | 40-140 | 250-442 | 5-17 | 18-22 |
| <i>Cleome gynandra</i> L. | 4-8 | 10-13 | 5-10 | 6-19 | 8.7 | 130-180 | 262-434 | 11-15 | 15-20 |
| <i>Amaranthus</i> spp. | 4-8 | 45 | 4-5 | 5-10 | 10.7 | 90-160 | 480-800 | 5-15 | 11-15 |

*Weeks from sowing to harvest.

Source: Abukutsa-Onyango, 2003; Gogo et al., 2016.

Table 2.2: Postharvest losses, storability and treatments of selected Kenyan African indigenous leafy vegetables (AIVs).

| Scientific name* | Shelf life (days)** | Quality loss symptoms | Storage temperature (°C) | Postharvest treatment*** |
|-------------------------------------|---------------------|--------------------------|--------------------------|--------------------------|
| <i>Vigna unguiculata</i> (L.) Walp. | 3 | wilting, yellowing, rots | - | Yes |
| <i>Solanum scabrum</i> L. | 3 | wilting, yellowing, | - | Yes |
| <i>Gynandropsis gynandra</i> L. | 4 | wilting, yellowing, | - | Yes |
| <i>Amaranthus</i> spp. | 3 | wilting, yellowing, | 5 | Yes |

*The AIVs are marketed in the open and closed markets (ambient conditions) as well as supermarkets (cold storage) and consumed fresh.

**At ambient conditions, all listed ALVs can only keep for one day. Shelf life indicated is for fresh AIVs.

***Most common postharvest treatments (preservation) are traditional (e.g. blanching, fermentation, drying).

Source: Gogo et al., 2016; Onyango and Imungi, 2007.

According to Smith and Eyzaguirre (2007), there is a need to develop and promote appropriate handling and processing technologies to minimize postharvest losses and ensure quality and safety during the supply chain in rural, peri-urban and urban centres. Just like other leafy vegetables, AIVs should be prepared for market or preservation as soon as possible after harvesting. Since these vegetables are highly perishable, the likelihood of spoilage increases rapidly after harvest, as time passes. Therefore, these vegetables need to be transported to a nearby shade or cold store



within the shortest time possible. Majority of farmers producing these vegetables are resource limited (Yadav and Sehgal, 2002); hence cannot afford conventional cold stores. Most farmers normally prepare their vegetables under a tree shade or ordinary (traditional) stores. Some farmers sprinkle cold water on the vegetables in order to quickly remove field heat and maintain freshness for a longer time. At this point, these vegetables are sorted, cleaned and packed using farmer and consumer specific requirements. Cleaning (or washing) is necessary to remove any dirt or residues. Unfortunately, many farmers forget to dry leaves after washing and therefore encounter higher prevalence of disease development, especially moulds (Gogo et al., 2016). However, at supermarkets these vegetables are stored in cold shelves at about 5-10 °C together with other vegetables and fruits. Knowledge of low temperature storage of AIVs along the supply chain is still limited. Very low temperatures (< 5 °C) have been reported to cause chilling injury to AIVs (Nyaura et al., 2014). Mixing of these vegetables with ethylene producing fruits and vegetables (as in the case of most markets and supermarkets) will hasten their deterioration rate. In most cases, if these vegetables are not sold within 24 hours after harvest, the likelihood of deterioration is imminent. In the case of the remaining AIVs from the previous sale, some farmers have tried to sprinkle water on the vegetables and leave them in the open overnight. However, problems of disease development and thus microbiological contamination still hamper their effort. Moreover, nutritional quality is also highly affected by the different postharvest temperatures. For example, it has been demonstrated that ascorbic acid declined by 88% in vegetable amaranth when kept at room temperature (20 °C) after 4 days of storage while the lowest loss was observed at 5 °C (55% loss) after 23 days of storage. Based on this study, Nyaura et al. (2014) suggested that shelf life extension and nutrient preservation of vegetable amaranth can be achieved through storage at temperatures of 5 °C. These problems are further aggravated by the increasing dietary needs of the ever growing Kenyan population. Since majority of the Kenyans depend on AIVs as a source of nutrition and income, there is need for urgent intervention.

2.2. Emerging pre- and postharvest technologies for fresh vegetables

There is an increased awareness of quality attributes of vegetables including freshness, colour, texture, flavour, nutritional content, health promoting secondary compounds and food safety among consumers and traders. Modification of existing pre- and postharvest techniques and/or the adoption of novel technologies that allow for production of safe and high quality products are strategies undertaken to meet these increasing customer demands (Gonzalez and Barrett, 2010). There are evidences on the biological benefits associated with a particular novel vegetable pre- and postharvest treatment technique, for example, impact of the technology on vegetable like in this case electric current and UV-C (Figure 2.1). In this section, we focus on emerging pre- and postharvest treatment technologies, hereby exemplarily demonstrating pre-harvest and postharvest application of electric current, UV-C irradiation and their impact on morphological, physiological and biochemical changes on fresh vegetables.

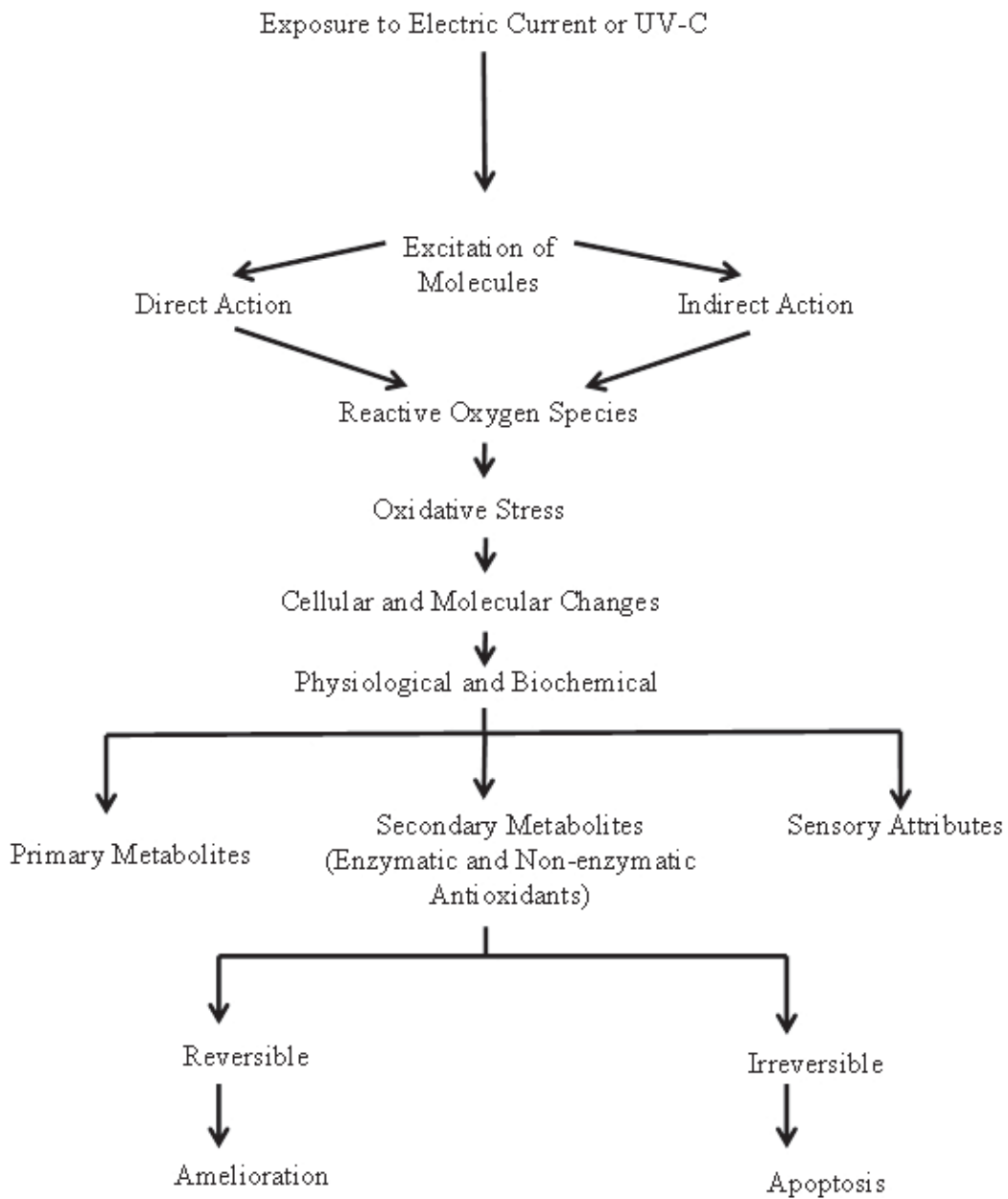


Figure 2.1: Schematic representation of the effect of electric current or UV-C on molecular, biochemical and physiological changes in vegetables.

(Adapted with slight modifications from: Kasote et al., 2015; Rohanie and Ayoub, 2012).



2.2.1. Electric Current (fields): Impact on morphology, physiology and bioactive chemical compounds in vegetables

The theory of electro hydrodynamics can be invoked to explain the physical action of ions in a substrate resulting from ionisation in an electric field. Thus, the familiar Coulomb force of the product of field strength and charge density becomes the principal driving force for physical action of the ions. When the ions are subjected to electric fields, the charged particles will accelerate. The kinetic energy gained by the particles is partly spent in ionising other molecules via the Ramsauer–Townsend effect (Bailey and Townsend, 1921) and partly in colliding with neutral molecules in the drift region of the fluid medium in which they travel. This results in collisions, while the momentum is transferred to the molecules hence frictional resistance. This produces the ion drag phenomenon with the associated electric force when the substrate mass between the electrodes moves towards the plate. The generation of vortex motions by the electric current with associated enhancement in heat transfer from metallic plates has been well documented in fluid mechanics (Jones, 1978). Therefore, the presence of an electric field affects molecular bonding of a material, including vegetable tissues. Furthermore, Sidaway (1966) showed the differences of plant response towards the negative and positive signs of electrostatic fields. It is also demonstrated that electric fields accelerate mass transfer by affecting membrane permeability properties (Rastogi et al., 1999). The application of electricity with the voltage of ≤ 2 V is reported to allow ions and molecules crossing the cell membranes (Soliva-Fortuny et al., 2009; Weaver and Chizmadzhev, 1996). This effect was also confirmed by Angeschbach et al. (2000) who reported formation of pores across the cell membrane when a transmembrane potential of 1.7 V was reached in potato (*Solanum tuberosum* L.) tissue. They demonstrated that, conductive channels that occurred across the membrane resealed again shortly after electric current application and the cell membrane recovered its electrical insulating properties. Such a phenomenon offers numerous possibilities to induce targeted stress reactions in plant systems or cell cultures, whereas cells regain their vitality and metabolic activity. Therefore, the application of electric current, especially at low intensity (< 20 V) does not necessarily result in irreversible cell rupture as it is mostly perceived. In view of the urgency for increased food quality production in our century and in the future, it seems appropriate to bring this subject to the attention of researchers and others who would seek to apply the technology.

2.2.1.1. Pre-harvest application effects of electric fields on vegetables

The application of electricity can affect growth and development of plants to a great extent. This little-known technology, called electroculture, when applied to growing plants can affect growth rates, yields, and crop quality (Pohl, 1978). Studies also indicate that electroculture does not only protect plants from weeds, diseases, insect pests, and frost but also reduces the requirements for fertilizer or pesticides (Pohl, 1978). This technology has been applied to plants, seeds, soil, water, and nutrients (Diprose et al., 1984). Plants also have electric fields, which seem to be a vital part of their physiology (Scott, 1967). For example, an electrogenic proton pump helps in the regulation



of cytoplasmic pH, and the active transport of mineral ions, hormones, or organic metabolites (Spanswick, 1981). Small changes in intracellular pH may act as a regulatory signal for certain critical steps in cell activity (Kurkdjian and Guern, 1989). Internal electric fields of plants may be affected by externally applied electric fields (Murr, 1965). Studies demonstrated that electric fields affected growth and development as well as physiological and biochemical compounds of vegetables (Table 2.3). Electric fields can significantly improve growth of vegetables (Costanzo, 2008; Murr, 1965, 1964; Ward, 1996). This could be attributed to the changes in the biophysical mechanisms. Studies have also demonstrated effects of electric fields on mineral element uptake and accumulation on vegetables (Dannehl et al., 2012; Murr, 1965, 1964; Ward, 1996; Zvitov et al., 2003). This may be attributed to changes in the enzymatic activities as well as electrolyte leakage as a result of membrane permeability (Gürsul et al., 2016). In electric current field studies, electrodes are used and therefore possibility of heavy metal accumulation. However, studies demonstrated that using metals as electrodes, resulted in slight increase in heavy metals but did not have toxic effects as the contents were within the legal regulations for human consumption (Dannehl et al., 2012). In other studies, physiological activities on vegetables were enhanced by electric fields (Montavon et al., 1987; Morris, 1980; Zvitov et al., 2003), attributed to stress related metabolic activities. Electric fields have effects on enzymatic and non-enzymatic composition of secondary plant compounds (Bratton and Henry, 1977; Dannehl et al., 2012; Dannehl et al., 2009; Gürsul et al., 2016; Montavon et al., 1988). The authors suggested that the physiological stress may result in changes in plant secondary metabolism.

Table 2.3: Summary of studies on pre-harvest application effects of electric fields on vegetables during production.

| Vegetable | Type | Intensity | Effects | Reference |
|---|------|--------------------------------|---|-----------------------------|
| <i>Glycine max</i> (L.) Merr. | AC | 1800-3600 V m ⁻¹ | Increased seedling length. | Costanzo, 2008 |
| <i>Zea mays</i> L., <i>Phaseolus vulgaris</i> L. | AC | 50 KV/1 mA | No effect on N and P levels and increased dry weight, Fe, Zn, and A contents. | Murr, 1964, 1965 |
| <i>Solanum lycopersicum</i> L. | DC | 6 V | Increased in dry weight, Mg, Ca, and N contents. | Ward, 1996 |
| <i>Cucumis sativus</i> L., <i>Phaseolus vulgaris</i> L., <i>Commelina communis</i> L. | DC | 5-20 V/0.5 kV cm ⁻¹ | Increase in stomatal opening, higher contents of K, Na, Ca and S in leaves. | Zvitov et al. 2003 |
| <i>Solanum lycopersicum</i> L. | DC | 3-7 µA | Increased peroxidase activity and indoleacetic acid in leaves while lower levels were observed in the roots. | Bratton and Henry, 1977 |
| <i>Pisum sativum</i> L. | DC | 9.0 V/15-20 µA | Inhibited accumulation of indoleacetic acid (auxin). | Morris, 1980 |
| <i>Spinacia oleracea</i> L. | DC | 10 V/12.5 µA | Increased glucose-6-phosphate dehydrogenase and peroxidase activity. | Montavon et al., 1987, 1988 |
| <i>Raphanus sativus</i> L. | DC | 200-1000 mA | Increased the phenolic compounds in tubers and roots. | Dannehl et al., 2009 |
| <i>Lepidium sativum</i> L. | DC | 200-1800 mA | Induced biosynthesis of chlorophyll, proteins, and phenolics, no significant accumulation of heavy metals was observed. | Dannehl et al., 2012 |
| <i>Solanum lycopersicum</i> L. | DC | 600-1200 V cm ⁻¹ | Increased phenylalanine ammonia lyase activity, phenolic compounds and cell membrane permeability. | Gürsul et al., 2016 |

2.2.1.2. Postharvest application effects of electric fields on fresh vegetables

Electric current has been reported to preserve or improve quality in vegetables even after harvest (Kharel et al., 1996). The studies on postharvest effects of electric field on fresh vegetables include biosynthesis of ethylene (Inaba et al., 1991), accumulation of bioactive compounds (Dannehl et al., 2011), morphological and physiological changes (Dymek et al., 2014; Kharel et al., 1996) (Table 2.4). Atungulu et al. (2004) reported that mechanism by which electric current could affect produce quality includes changes in physiological and secondary metabolism as well as inhibiting