# THE EFFECTS OF *Cuscuta campestris* Yunck INVASION ON TEA GROWTH PARAMETERS, QUALITY AND SOIL NUTRIENT UPTAKE IN NANDI, KENYA

**BY**

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**A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN ENVIRONMENTAL BIOLOGY OF THE UNIVERSITY OF ELDORET**

**OCTOBER, 2022**

## DECLARATIONS

**Declaration by student**

I declare that this thesis is my original work and has not been submitted for award of a degree in any university or institution of higher learning except where acknowledged.

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## DEDICATION

This work is dedicated to my supervisors and my family.

## ACRONYMS AND ABBREVIATIONS

| | |
|---|---|
| Ca | Calcium |
| Cu | Copper |
| Fe | Iron |
| G | Gram |
| GDP | Gross Domestic Product |
| ICP | Inductively Coupled Plasma |
| K | Potassium |
| Kgs | Kilograms |
| Mg | Magnesium |
| Mn | Manganese |
| Na | Sodium |
| P | Phosphorus |
| pH | Potential of hydrogen |
| TRI | Tea Research Institute |
| Zn | Zinc |

## ABSTRACT

Major agricultural crops around the word face problem of parasitic infestations. Almost everywhere in the world, there are about 200 species of parasitic plants of the genus *Cuscuta*, family of Cuscutaceae also known as dodder. It is one of the most damaging parasitic plants for the worldwide agricultural broad-leaved crops. Tea (*Camellia sinensis*) bushes in Nandi County have been under invasion by *Cuscuta campestris* since 2013 posing a major threat to the existing tea clones, human livelihood and ecosystems in general. *Custuta* spp. depletes nutrients and other soluble components thereby stressing tea plant growth finally resulting in yield losses. This study was conducted in Nandi County to assess the effect of *Cuscuta campestris* on tea clones' number of leaves, leaf area, trunk diameter, dry weight and quality. Biophysical/ biochemical factors that enhance *C. campestris* invasion on tea were also assessed. Complete randomized design was used in the experiment. Tea clones in potted tea plants in a greenhouse were infected with *C. campestris* twigs collected from infected tea clones in Nandi tea estates. Data analysis was performed in SPSS version 21 where differences in mean between infected and control were done using t-test. Chi- square test was use to analyses the differences between observed and expected percentages frequencies. From the findings, *Cuscuta campestris* affected harvest biomass in all the 6 clones (TRFK 430/90, TRFK 306/1, TRFK 31/8, TRFK 301/4, TRFK 303/577 and EPKTN14-3 and their respective controls), trunk diameter of clone TRFK 306/1, leaf area of clones TRFK 31/8 and TRFK 301/4 compared with the control. Among the six clones tested, only TRFK 306/1 had a significant difference (between infected (1.04±0.15) and control (0.7±0.04) for trunk diameter. Mean leaf area for the infected TRFK 306/1 clone (40.33±6.50) was significantly different (t=-3.0110, p=0.03951) from its mean control (46.00±14.00). For the TRFK301/4 clone, mean leaf areas (29.33±2.08) was significantly different (t=-2.94174, p=0.0423) from the mean leaf control (34.33±2.08). Mean number of infected leaves of 303/577 clone was highest (73.00±5.29) followed by TN14-3 (61.66±4.72) with insignificant differences from mean control. Differences in mean control and mean infected of harvest biomass including above and below ground tissue were assessedAll the six clones showed a significant difference compared with their controls. Biochemical quality of tea clones was not affected by *C. campestris*, including GC ($p>0.05$), EGC($p>0.05$), +C($p>0.05$), EGCg ($p>0.05$), EC($p>0.05$), GCg ($p>0.05$), ECg ($p>0.05$), Cg($p>0.05$), GA($p>0.05$), and Caffeine($p>0.05$). *Cuscuta campestris* invasion had no effect on biochemical soil components. In conclusion, harvest biomass of all tea clones was affected by *C. campestris* infestation with infected clones having significantly higher biomass. In addition, *C. campestris* does not affect nutrients uptake by the host plant. Cross breeding of clones especially TRFK306/1 with other more resistant clones would probably make TRFK306/1 more resistance to attack.

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## ACKNOWLEDGEMENTS

I thank our Almighty God for the gift of life and good health. My strongest appreciation goes to my supervisors Dr. Shem M. Mwasi and Prof. Vincent Sudoi of University of Eldoret for their constant guidance during the writing of this thesis. My appreciation also goes to Dr. Evelyn Cheramgoi of Kericho tea research Foundation for utmost contribution to this research.

# CHAPTER ONE

# INTRODUCTION

## 1.1 Background of study

Parasitic plants exist almost everywhere in the world with about 400 species. Some of parasitic plants have become weeds posing a major a threat to major crops including grains and forage legumes (Rubiales and Heide-Jorgensen, 2011). There are about 200 plants of the genus *Cuscuta,* that belong to the family of *Cuscuta*ceae. *Cuscuta campestris* is known to be a serious weed where broad leaved crops are grown as perennials. This parasitic plant species is leafless twined sprawling thin vine that grows over a host plant. Most *C. campestris* species have been introduced to various regions of the world together with seeds of commercial crops, especially legumes such as alfafa (*Medicago sativa*) and clover (*Trifoliate* species) and therefore are widely distributed throughout the temperate and tropical zones (Kaiser, 2015). The parasitic weed has been in existence in Kenya from the year 2007 and has been observed to attack mostly shrub and trees such as Mauritius thorn, K-apple, *Bougainvillea*, Mango, Nandi flame, Loquat, *Acacia* and Tea (Bore *et al*., 2014).

In Nandi tea plantations, *C. campestris* has posed threat to tea trees since 2013, human livelihood and ecosystem in general (Kerich, 2014). *C. campestris* invasion may cause 50%-98% yield loss (Zharasov, 2009). *Cuscuta* parasitic plant is able to withdraw water, carbohydrate and other soluble materials (polyphenols and 1 lavonoids) from the susceptible host by twining around the host and penetrating the stem via the haustoria into the vascular bundles (West wood *et al*., 2012). *Cuscuta* spp. operates as a "super-sink" when attached to a host. It results to heavy loss due to withdraw of host resources such as water and nutrients resulting into heavy loss in terms of yield. Hard coated seed of *Cuscuta*

*campestris* make it extremely difficult for farmers to control. Hard coat ensures the parasite seed remain dormant yet viable for a quite a period of time. *Cuscuta campestris* also obtain minerals such as sodium, calcium and magnesium through the phloem tissue. *C. campestris* also tend to be highly concentrated in the phloem than the xylem according to a study by Kaiser *et al*. (2015). Competition for carbon between the parasite and host depends largely on the relative sink strength of the parasite and degree of autotrophy of the parasite (Press and Phoenix, 2005).

*Cuscuta campestris* parasitic plant invasion in Nandi tea plantations may be due to disturbances such as fires, floods, grazing, long periods of rain, human modifications of the habitat or fluctuations in soil nutrients that create avenues for invasion (David, Arulmoli, and Parasuraman, 2016). Using *C. campestris* tolerant clones would be the best method of controlling dodder and prevent losses in tea due to stress that affect growth and reduce yields and possible effect on quality.

Extracts from tea trees (*Camellia sinensis*) are used globally to make a variety of every day beverages because of their therapeutic properties. Biological components of tea include polyphenols, amino acids, saponins, fluorides, caffeine, vitamins, minerals, fragrant and trace elements. The antioxidant and free radical scavenging abilities of catechins and flavonoids in tea play a great role in prevention of cardiovascular diseases, chronic gastritis and some types of cancer diseases (David, Arulmoli, and Parasuraman, 2016). Since parasitic plants obtain part or all of their nutrients from host plant, they significantly influence hosts' community composition and dynamics through lost biomass (Lanini. 2014).

Despite the ecological and economic significance of this parasitic weed, its effects on tea clones' growth parameters, yield, catechins and soil components and the factors that drive its invasion in tea are poorly understood

### 1.2 Statement of problem

Nandi tea plantations have been under *C. campestris* attack since 2013. Continued *C. campestris* spread is a major threat to the already established and existing tea clones in Nandi area. Parasitic weed invasion has led to both direct and indirect effects within the host tea plants. These include reduction in yield as the parasite depletes nutrients and other soluble components weakening the host (Rana andand Rana, 2016). This problem has persisted and no research has been carried out to determine the effect of *C. campestris* effects on tea clones in the region.

### 1.3 Justification of the study

Tea is the main economic crop in Kenya (Waithaka, 2006). Tea is also consumed locally and internationally because of its multiple preventive and therapeutic effects from the polyphenols it contains which are affected by dodder invasion (Kanwar, 2012). Therefore, it is important to properly manage and control any parasitic invasion on it. Control of *C. campestris* parasitic invasion is extremely difficult hence the need to determine which tea clones are most susceptible to attack and overall effect of *C. campestris* on growth parameters of tea and how it affects nutrients uptake.

### 1.4 Significance of the study

The knowledge on the effects of *C. campestris* invasion on tea clones will help in development of tea clones that are tolerant to *C. campestris* attack. And consider

development of clones that behave differently under different soils and moisture regimes. This would help to improve yield and quality.

### 1.4 Main objective

To assess the effect of *Cuscuta campestris* on tea clones' growth, yield quality, nutrients uptake and the biophysical factors that influence invasion in Nandi area

### 1.4.2 Specific objective

i. To determine the effect of *Cuscuta campestris* on tea clones' growth and yield.
ii. To determine the effect of *Cuscuta campestris* on tea catechins.
iii. To evaluate the effect of *Cuscuta campestris* on uptake of soil nutrients by tea clones.

### 1.5 Research hypothesis

i. $H_{O1}$: *Cuscuta campestris* does not have any effect on tea clones' growth and yield in Nandi area.
ii. $H_{O2}$: *Cuscuta campestris* does not have any effect on tea catechins of tea clones in Nandi area.
iii. $H_{O3}$: *Cuscuta campestris* does not have any effecton uptake of soil nutrients by tea clones.
iv.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 Introduction

### 2.1.1 Classification of *Cuscuta campestris*

*Cuscuta campestris* is among parasitic plants belonging to the family of Convolvulaceae that comprises about 200 species of obligate holoparasitic plants (Garcia, 2014). There are 3 sub-genera based on morphology of the styles and the stigma. They are sub-genera monogyna, with one style that is partially or wholly joined to the stigma lobes (Gramza-Michałowska, 2014). They favor trees and shrubs as hosts (Dawson *et al*., 1994). The sub-genera Glammica which is the most diversified and characterized by two styles and capitated stigma (Guerra and Gacia, 2004). The genera *Cuscuta* with two styles and elongated stigma (Guerrala and Gacia, 2004). *Cuscuta* thrives well in humid and warm climate where they cause crop losses (Kaiser, 2015). *Cuscuta* is widely distributed across the globe. There are five native species in Europe and the most common in the five is *C. europeae* (Soukand, 2013). The species that attack cultivated crops are *C. pentagonia* and *C. campestris* which are widely distributed and have a variety of host plants. About 25 crops are known to be attacked by *Cuscuta* (Lanini and Kogan, 2005).

### 2.1.2 Life cycle of *Cuscuta* spp

The life cycle of *Cuscuta* spp. begins with seed germination. The seedlings emerge without cotyledons or leaves and with reduced leaf-like structures that easily degenerate (Sarić-Krsmanović, 2020). Germination of the seedlings occur at temperatures of 30 $^{o}$C-33 $^{o}$C. *Cuscuta* seedling must attach to a host plant in order to survive and complete its life cycle (Westwood *et al*., 2009). Ability to recognize host plant volatiles as chemo attractants

enables *Cuscuta* spp. to find and attach to the host (Hegenauer, Körner, andand Albert, 2017). Contact with a stem make the seedling to twin and lose contact with the soil surface and begins to spread throughout the host plant (Kaiser *et al*., 2015). Contact stimulates the development of prehaustoria that forms a connection of vascular bundles between the host and the parasite, the parenchyma and meristem cell of *Cuscuta* spp. differentiates into secondary meristem (Kaiser *et al*., 2015). Hyphae is sent into the sieve cells of host and cytoplasmic connection develops between the parasite and the host (Yoshida *et al*., 2016). Materials such as water, proteins, nitrogen compounds and viruses are conducted through this connection. Flowering and seed formation by the parasite follow during maturity. The host plant dies before maturity because the parasite is able to complete its life cycle earlier than the host (Kaiser *et al*., 2015). *Cuscuta* spp. flowers are numerous, tiny and white-pink and form in small clusters along the stems, depending on the species and location. Each flower forms a small globular seedpod with 2-4 seeds with rough coats that are able to survive for more than 20 years (Sharib, *et al*., 2020).

The presence of *Cuscuta* spp. is manifested by its twining stems and tendrils. It is known to attack all the plant stages; the seedling stage, vegetative growing stage, flowering stage, and fruity stage causing serious damage to the leaves, the whole plant, and fruits/pods. The seeds can be distributed through irrigation; infested livestock fodder crops and contaminated seeds of cultivated crops. Survival of *Cuscuta* spp. is through siphoning of soluble nutrients from the hosts sink through the phloem connection (Hodzic, 2021). *C. campestris*, also known as field dodder which originated from North America, is the most common and widespread species in the world especially in the tropics and sub-tropic regions (Garcia, 2014). It is totally dependent on the host depleting nutrients and

subsequent death of the host (Dawson *et al.*,1994). *C. campestris* is a major threat to economic crops (Hodzic, 2021).

### 2.1.3 *Cuscuta* spp. hosts

Due to the reduced amount of chlorophyll, *Cuscuta* spp. parasitic plants may be able to carry out insignificant amounts of photosynthesis or unable to carry out photosynthesis (Rubin andand Artikhovskaya, 2013). *C. indecora, C. campestris* and *Cuscuta proximate* are known to carry out small quantities of photosynthesis (Wiebe, 2011). *Cuscuta* spp. has a wide range of hosts mostly dicotyledonae such as alfafa, legumes and some horticultural crops such as vegetables, carrots, clover, onion, potato, water melon, and sugar beets (Rana andand Rana, 2016). The seeds may persist in the soil for long seasons from winter and germinate in the following spring. In humid and warm climates, it depends on crops resulting to crop losses (Lanini andand Kogan, 2005). Field dodder may cause about 50%-90% yield loss (Parker, 2012). The common host for *C. campestris* are grass and agricultural crops (Baráth, 2021).

## 2.2 Effect of *Cuscuta* spp. on hosts' yield

Shen *et al*. (2011) and Li *et al.* (2017), have illustrated the effects of *Cuscuta* spp. on *Mikania micrantha* in China from flowering stage to the host death. In other host plants, *Cuscuta* spp. has led to premature fruit drop, yellowed or dead leaves as well as whole plant early senescence. Some host plant to *Cuscuta* spp. produce terpenoids α-pinene, β-phellandrene and β-myrcene as chemical cues that serve as chemo-attractants (Kaiser *et al*., 2015).

According to Tadesse *et al*. (2015), *Cuscuta* spp. is able to redirect the flow of sugar, amino acids and other solutes from hosts' destination into the parasite. The host increases its rate of photosynthesis making it susceptible to water loss. *Cuscuta* spp. attaches and parasitizes a host with high nutritional quality host and reject hosts with poor nutrition (Touchette, Feely andand McCabe, 2021). Field dodder attaches to stems and leaves of field crops, vegetables and ornamentals, throughout in most agricultural regions globally (Lanini andand Kogan, 2005). The damage of dodder to the host varies from moderate to severe depending on the position of haustoria attachment and the number of twigs (Alakonya *et al*., 2012). Most dicotyledonous crops have been reported to be attacked by *Cuscuta* especially legumes (Tadesse *et al*., 2015). *Cuscuta campestris* has been shown to mount severe attack on Lucerne because both seeds are small hence difficult to separate them and it also depend totally on the host for survival (Lanini andand Kogan, 2005).

In China, *Cuscuta* attack led to yield losses in soya beans and tomatoes (Gunathilaka *et al*., 2018). Yield reduction in the year 2009 from *Cuscuta* attack in India were 60-65% in chilies, 31-34% in green gram (Vigna radiate), 87% in lentils, 86% in chicken pea, 72% in tomatoes and 60-70% in alfalfa (Lanini andand Kogan, 2005). *Cuscuta* parasitizes and shades out the host, reducing vegetative parts and a decrease in biological biomass (Fathoulla andand Duhoky, 2008). It has also been reported that dodder infection may cause 50% to 98% yield loss (Zharasov, 2009). Since parasitic plants obtain part or all of their nutrients from the host plant they significantly influence community composition and dynamics through reduction in lost biomass (Shen *et al*., 2005).

Not all plants are susceptible to *Cuscuta* spp. attack. In India, *Cuscuta* spp. is a serious agricultural threat to green gram (*Vigna radiata*), oil seed niger (*Guizotia abyssinica*),

pulses (black gram (*Vigna mungo*), linseed (*Linumusita tissimum*) among others. A few plants such as tomatoes are able to fend off *Cuscuta* spp. Many monocotyledonous are known to develop resistance against *Cuscuta* spp. attack because of anatomical reasons such as arrangements of vascular bundles and incompatibility of signals important in the formation of interspecies connection of vascular strands (Benaiche, 2016). *Cuscuta australis* however is known to actively attack monocotyledonous plants. Active resistance against *Cuscuta* attack is shown by the families of Gossypium, Malvaceae, hirsutum and rosa-sinensis (Sahu *et al*., 2012). Cultivated crops such as tomato (*Solanum lycopersicum*) are known to develop resistance against *Cuscuta reflexa* attack (Sahu *et al*., 2012). After penetration by the haustoria the host forms a wound tissue on the area of infection preventing the establishment of cytoplasmic connection between the host and the parasite (Sarić-Krsmanović, 2020). Day 3-5 after haustorial contact the hosts epidermal cells elongates strongly due to an increase in auxins production in both the tomato epidermal cells and the parasitic prehistorium, hence bursting of the hosts epidermal cells on the contact site. Death of the parasite follows 15 days later (Shen *et al*., 2011).

### 2.3 Effect of *Cuscuta* spp. on the hosts' quality

*Cuscuta* is a serious problem in forage legumes, such as clovers, alfalfa, and niger. Other leguminous crops that play host to *Cuscuta* spp. included chickpea, linseed (*Linumusitatis simum*), sesame (*Sesamum indicum*), lentil and pea (*Pisum sativum*), pigeon pea (*Cajanus cajan*) among others. *Cuscuta* spp. also parasitizes numerous species of dicotyledonous weeds and wild plants. *Cuscuta* can parasitize asparagus (*Asparagus officinalis*) and onion (*Allium cepa*), which are monocotyledonous crops, but grasses and grains (Poaceae) are usually not parasitized (Sahu *et al*., 2012).

*Cuscuta* spp. attack to the host ranges in severity based on its species and the species of the host and the time of attack (Qasem, 2011). By debilitating the host plant, *Cuscuta* spp. decreases the ability of plants to resist diseases emanating from viruses and spreading diseases from one host to another (Sahu *et al*., 2012). This is of economic concern in agricultural systems, where an annual drop of more than 10% yield can be devastating to the farmers (Qasem, 2011). There has been an emphasis on dodder vine control in order to manage plant diseases in the field.

*Cuscuta* spp. is reported as a gall-inducing agent, and well-marked host responses sequential to haustorial invasion (Qasem, 2011). *Cuscuta* spp. has been listed as a serious parasitic weed in several countries. Land use practices such as crop rotation and planting of unfavorable host to *Cuscuta* spp. has led to minimum areas of attacks thus rarely a major weed over large areas (Sahu *et al*., 2012).

### 2.4 Biophysical and environmental factors that enhance *Cuscuta* spp. invasion

#### 2.4.1 Temperature

According to Sahu *et al*., (2012), plants growing in warm environment produce higher amount of dry matter than those in cooler areas and shoot growth increases with increase in altitude. Temperature plays a significant role in dodder seed germination (Johnson *et al*., 2013). According to Meulebrouck (2008), *Cuscuta* spp. seeds require cold temperatures to break seed dormancy. Seed germination occurs and continuous at temperature between 0$^{o}$c to 38 $^{o}$C with optimum temperature range of 30 $^{o}$C to 33 $^{o}$C (Lanini andand Kogan, 2005). In a study carried out on *Cuscuta campestris* by Sahu *et al*., (2012), air temperature plays a role in the first emergence of the seedlings with the average air temperature above 18 $^{o}$C. Meulebrouck (2008) found out that *Cuscuta* seed germination is fastest at high temperature

and lowest at low temperature. The favorable temperature for *Cuscuta* spp. germination and emergence is in the range of 15 $^{o}$C to 38 $^{o}$C, with optimum of 30 $^{o}$C (Meulebrouck (2008).

Li *et al*. (2017) observed that soil chemical components can influence the germination of *Cuscuta* seed. In addition, 60°F and above in green house experiments were favourable for *Cuscuta* seed establishments (Meulebrouck, 2008).

### 2.4.2 Humidity

According to Johnson (2013), *Cuscuta* spp. seed germination was enhanced in humid conditions while haustoria formation is best in low humidity (20.0%). Humidity affects the growth and health of most parasitic plants and majority thrive in very humid tropical locations (Waithaka *et al*., 2006).

### 2.4.3 Light

Light can have profound impacts on *Cuscuta* spp. development. Aspects such as light quantity, exposure duration and spectral wavelengths all influence dodder photomorphogenesis, or the light-induced changes within the plant. Sunlight contains a large amount of electromagnetic radiation in the red (620-700 nm), far-red (700-800 nm), and infrared (800 nm -1 mm) portions of the spectrum, all of which pass through the leaf canopy and enter several millimeters into the soil (Albert *et al*., 2008). Longer wavelengths of light travel deeper into the soil than shorter ones, causing a decrease in the ratio of red to far-red light (R:FR) with increasing soil depth (Touchette, Feely andand McCabe, 2021). Germination of many plant seeds can be stimulated when red and far-red light is detected by the protein photoreceptor phytochrome (Takano *et al*., 2009). When buried seeds (which

have the inactive form of phytochrome, Pr) approach the soil surface, they are exposed to higher R:FR and phytochrome is converted to the active form Pfr, triggering germination. While many weeds use light as a seed germination stimulus (Tadesse *et al*., 2015), it is thought that *Cuscuta* spp. seed germination is unaffected by light cues (Takano *et al*., 2009).

After germination, *Cuscuta* spp. seedlings emerge from the soil in the shape of a hook similar to an emerging bean hypocotyl (Lanini andand Kogan, 2005; Chang and Shen, 2011). Phytochrome is suspected to control hook opening a necessary step in the host acquisition process which leads to twining and haustoria formation (Lanini andand Kogan, 2005; because prolonged exposure to blue, red, and white light caused dodder hooks to open (Johnson, 2013). Ecological factors that influence host acquisition by the parasitic plant *Cuscuta campestris* while seedlings grown in darkness or infra-red light retained their hook shape (Lanini andand Kogan, 2005). For dark-grown seedlings, even a single flash of red light caused the hook to open.

Both quantitative and qualitative aspects of light can affect the circumnutating movements of plants (Susanti *et al*., 2015). Irradiance (the power of electromagnetic radiation per unit area) - which is a quantitative light trait -influenced *Arabidopsis thaliana* circumnutating: 97.5% of seedlings exhibited circumnutating under white fluorescent light at high irradiance levels (2300 Wm-2) while very few circumnutated at low irradiances (0.2 and 0.013 Wm-2) (Lanini andand Kogan, 2005). Wavelength-a qualitative trait-also affected *Arabidopsis* circumnutating: the period of circumnutating (amount of time to complete one rotation) was longer under continuous exposure to red light compared to white fluorescent light (Susanti *et al*., 2015).

Wavelength also affected rice (*Oryza sativa* L.) circumnutating: dark-grown wild type seedlings subsequently exposed to red light ceased circumnutating, and loss-of-function mutant plants (defective in phytochrome A) confirmed the participation of phytochrome in the circumnutation mechanism (Yoshihara andand Iino, 2005).

However, in dodder the effects of light on seedling circumnutating are less established. Lanini andand Kogan (2005); mentioned circumnutating occurs "without a terminal exposure to far-red but they do not incline sharply and twine. Omar *et al*., (2019) found circumnutating did not occur in dark-grown seedlings but began after seedlings were exposed to 16 to 24 hours of white fluorescent light. These observations suggest specific wavelengths of light may affect the circumnutating movements in dodders, but the details of such effects remain unknown.

Phototropism by dodder seedlings is influenced by specific wavelengths of light. *C. planiflora* seedlings exhibited positive phototropism toward far-red light in the presence of white fluorescent light but not in darkness. Smith *et al*., (2021) hypothesized that this observed phototropism toward regions of low R:FR was a method used by dodder seedlings to locate potential hosts since leaves of green plants absorb red but transmit and reflect far-red wavelengths, creating shady regions beneath the canopy with lower R: FR. Sunlight has a R:FR of about 1.1 while the area below the leaf canopy is enhanced with far-red wavelengths and therefore has a lower R:FR than sunlight (Ballaré andand Casal, 2000). Smith *et al*., (2021) tested the hypothesis proposed by Smith *et al*., (2021) by passing light through host leaves with different amounts of chlorophyll—which produced different ratios of R:FR—and then measured the growth response of *C. campestris* seedlings. Smith *et al*. (2021), found more than 60% of seedlings grew toward leaves with high levels of

chlorophyll (lower R:FR, 0.15) while significantly fewer seedlings grew toward the leaves with low levels of chlorophyll (higher R:FR, 0.23), thus supporting Rubin and Artsikhovskaya (2013) hypothesis.

Specific wavebands of light can also influence gravitropism in dodder seedlings and this action is believed to be regulated by phytochrome. When white-light-grown seedlings were cyclically exposed to red and far-red light, the seedlings that were terminally exposed to red light were negatively gravitropic while the seedlings terminally exposed to far-red light were positively gravitropic (Johnson, 2013).

Circumnutation of *Cuscuta* spp. seedlings can lead to twining or coiling of the parasite around an object (e.g., host plant), an event which must occur before parasitism can take place (Bolle, Koncz andand Chua, 2000). Seedlings of *C. pentagona* (Runyon *et al*., 2010), *C. indecora* (Marquardt andand Pennings, 2010) and *C. japonica* (Barilani *et al*., 2005) twined in darkness after pre-irradiation with white fluorescent light and a final brief exposure to far-red light or after continuous exposure to incandescent light; this effect was reversed by a final exposure to red light or continuous exposure to white fluorescent light. Blue light was even more effective than far-red light at stimulating the twining response (Bolle, Koncz andand Chua, 2000). Twining was not elicited by wavelengths between 500 - 700 nm (Runyon *et al*., 2010). Irradiance can also affect the twining response of dodders: seedlings did not twine when they were exposed to low irradiance levels (500 and 750 foot-candles of white fluorescent light followed by a brief exposure to far-red light) while twining was stimulated by high irradiance (1500 foot-candles of white fluorescent light followed by far-red light) Also, *Cuscuta campestris* and *Cuscuta indecora* seedlings did

not twine or produce prehaustoria in blue light at photon fluxes less than 1 μmol m-2 s-1 but did at photon fluxes greater than 4 μmol m-2 s-1.

Twining and prehaustoria formation are controlled by the actions of two photoreceptors: phytochrome and cryptochrome (Rubin and Artsikhovskaya, 2013). Phytochrome regulates the effects of red and far-red light, stimulating twining and prehaustoria formation after exposure to far-red light and inhibiting their formation after exposure to red light (Bolle, Koncz andand Chua, 2000). Cryptochrome regulates the stimulatory effects of blue light on twining and prehaustoria formation (Johnson, 2013).

The formation of mature haustoria is also a phytochrome-mediated response stimulated by exposure to far-red light or a mixture of far-red/blue light and inhibited by exposure to red light (Johnson, 2013). Additionally, haustoria did not form when *Cuscuta japonica* seedlings were exposed to white fluorescent light or kept in darkness (Bolle, Koncz andand Chua, 2000) even when tactile cues from objects (acrylic rods or glass plates) or a host plant were present (Barilani *et al*., 2005). These results suggest haustoria formation by *Cuscuta japonica* may be controlled by the combined effects of far-red light and tactile cues (Tadesse *et al*., 2015).

### 2.4.4 Wind

Host plant volatile odors provide an important signal for the host location process of dodder seedlings. However, environmental wind conditions (e.g., air turbulence, vortices, velocity, changes in directionality) could potentially be destructive to this odor-mediated foraging, although essentially nothing is known yet about this (Bolle, Koncz andand Chua, 2000).

### 2.4.5 Soil

When soil temperatures approach around 15.5 °C in the spring, native dodder begins to sprout at or very close to the soil surface. Independent of the host plant's impact, germination happens. A thin, twining stem that coils around any object, including host plants, is produced by the germinating seed.

*C. campestris* has been attacking the crop fields. The ongoing distribution of *C. campestris* poses a major danger to the established and existing tea clones in the Nandi region. The host tea plants have been affected by parasitic weed invasion both directly and indirectly. Reduced productivity brought on by the parasite's consumption of nutrients and other soluble host components is one of them (Rana andand Rana, 2016). The issue has persisted despite the lack of study into the effects of *Cuscuta* spp. in the region.

# CHAPTER THREE

# METHODOLOGY

## 3.1 Study area and experimental site

### 3.1.1 Study area

This study was carried out in Nandi tea estates which comprise of Nandi County, Taito and Savani tea estates (Figure 3.1). The tea estates consist of a total of 1,047 hectares of mature tea and processes 6,000,000kg of tea per annum out of which 95% is sold to the international market through auction and private sales (Titus andand Cheruiyot, 2013). This area was chosen for the study because it has been under *Cuscuta campestris* attack since 2013.

### 3.1.2 Experimental site

The experiment was carried out in a greenhouse of dimensions 30-feet wide, 96-feet long, 8-foot eave height and 5-foot roof height with temperatures of 23 -28 $^{o}$C at the Tea Research Institute in Kericho. The study was conducted from September 2018 to June 2019.

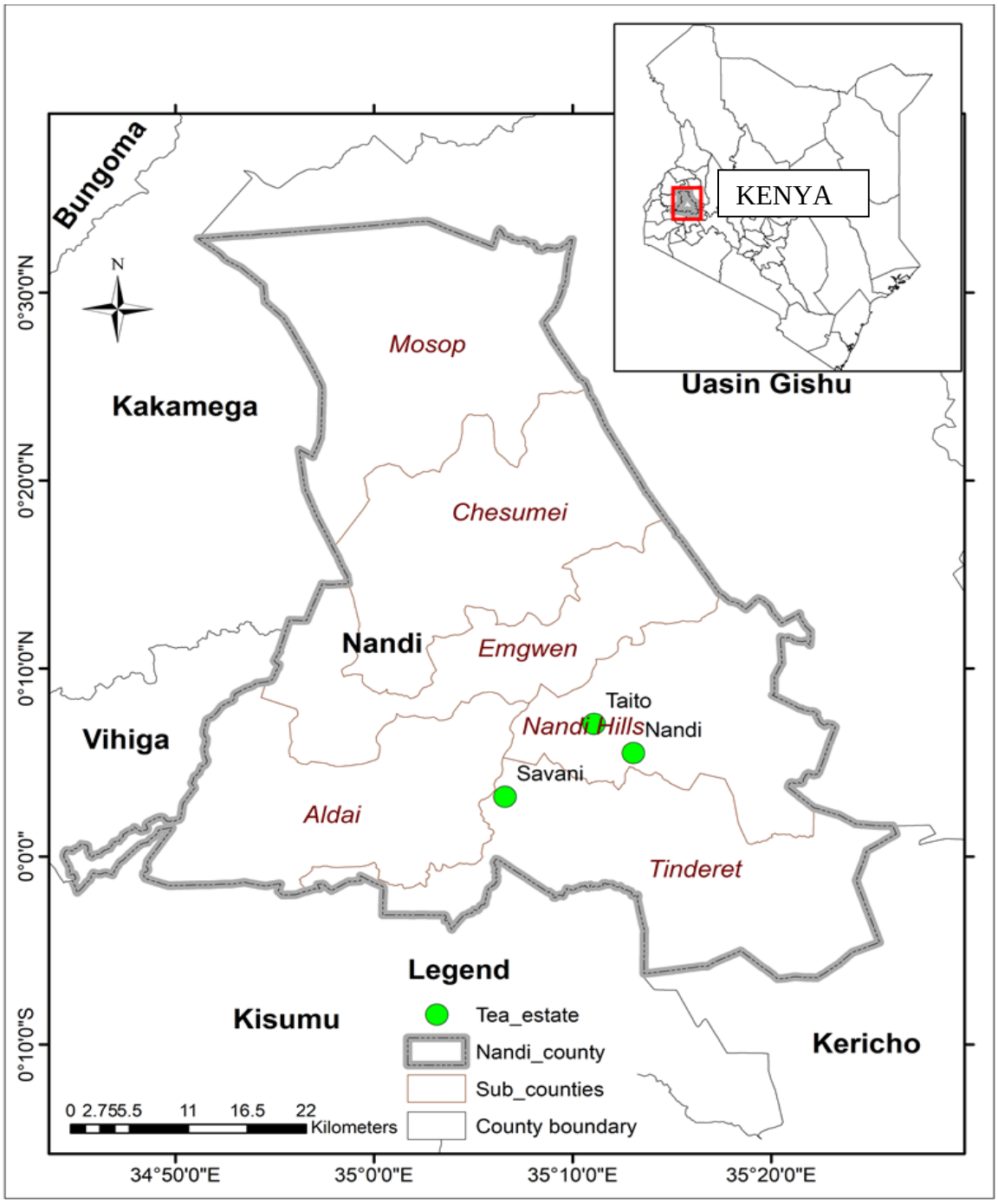


Figure 3.1: Map of study area

### 3.2 Research design

Completely randomized design was used in the subsequent arrangement of the treatments for the 6 experimental tea clone cuttings with their replicates used in determining the effect of *C. campestris* on the yield and quality of tea and in determining the biophysical factors that drive *C. campestris* attack on tea.

#### 3.2.1 Selection of experimental tea clones

On 20$^{th}$ December 2018, 6 tea clones were selected from Nandi Hills, Taito and Savani Hills tea estates using simple random sampling design. From the 42 tea clone varieties, 20 were selected for the study based on their distribution in the area, high quality and mostly cultivated. The 20 selected tea clone cuttings were assigned numbers 1-20 using pieces of paper which were folded, placed in a jar, shaken to mix them well then 6 were picked randomly as representatives for the study. The tea clone cuttings selected were TRFK 306/1, TRFK 430/90, TN14-3, TRFK 31/8, TRFK 303/577 and TRFK 301/4. The characteristics of the 6 tea clones are summarized in Table 3.1. Mature healthy shoot clone cuttings of single stem of uniform height of 4cm were raised in experimental pots with 75% of each pot filled with a mixture of top soil Nandi Hills, Taito and Savani Hills tea estates and 25% with sub-soil in the ratio of 3:1. The pots were then left for 2 months to allow sprouting and attain a height of 30 cm.

**Table 3.1: Characteristics of the 6 tea clones under investigation**

| Clone | Variety type | Special attributes | Status |
|---|---|---|---|
| TRFK 301/4 | Cambod type, local selection | High yield, high quality black tea and drought tolerant | Widely grown in Kenya, recently introduced to Tanzania and Rwanda |
| TRFK 31/8 | Assam type, local selection | High yielding acceptable black tea quality | Widely distributed in East Africa |
| TRFK 430/90 | Assam type, local hybrid | High black tea quality, high yielding | Recently released in Kenya for commercial use |
| TRFK 303/577 | Assam/China hybrid. op 6/8 | High black tea quality, high yield | Widely distributed in East Africa |
| TRFT 306/1 | Assam type of purple tea | Moderate yield, medicinal properties | Released for specialty tea in 2011 |
| TN14-3 | Assam type, local selection | Moderate yield, high black tea quality | Widely distributed in East Africa |

### 3.2.2 Infection of tea clones with *Cuscuta campestris* twigs

The 3 months old twigs of *C. campestris* were collected from infected tea clones at Nandi tea estates on 2nd February 2019. On 3rd February 2019 each of the 6 tea clones with 3 replicas were infected with 3 twigs of *C. campestris* of 27.94 cm length enough to twin around the branches. Infection was done through direct twining around the stem. This method of infection was chosen because chances of successful infection is high compared to infection using *C. campestris* seeds. A control for each experimental set up was also replicated 3 times. The pots were placed in the greenhouse maintained at temperatures of 23 ºC-28 ºC. Row to row and plant to plant distance was 1meter. Each pot was separated from the other using a mosquito net to restrain movement of *C. campestris*, and therefore no plant- plant interaction. Irrigation was done daily with 200 ml of water. Top dressing with a controlled amount of calcium ammonium nitrates fertilizer was also done. Weed control was done until the end of the experimental period. The greenhouse experiment for the treatments was conducted in randomized design each having 3 replications aimed at evaluating the effect of *C. campestris* on tea clones yield and quality. The growth parameters considered include the number of leaves, leaf area, trunk diameter and dry weight (yield). The performance of *C. campestris* and its effects on soil composition were also assessed.

**Table 3.2: Factorial arrangements of experimental pots for infected tea clones and controls**

| | | |
|---|---|---|
| TRFK31/8 | TRFK301/4 | TRFK303/577 |
| TRFK303/577 | TRFK303/577 | TRFK303/577 |
| TFRK430/9 | TRFKK306/1 | EPKTN14-3 |
| EPKTN14-3 | EPKTN14-3 | TRFK306/1 |
| TRFK301/4 | TRFK430/9 | TRFK301/4 |
| EPKTN14-3 | TRFK31/8 | TRFK430/9 |

### 3.3 Data collection and instruments

After two months, all the tea clones infected with *C. campestris* were well established. Throughout the experimental period, weed control was done when necessary, watering was done daily at 9.00 hrs except those representing the dry watering regime that received water thrice in a week at the same time. After 6-months period, data for the physiological and biochemical parameters was collected.

Laboratory analyses of catechins were conducted using the procedure also used by Crew (2015). Agilent 1260 HPLC series made at 278 nm wavelength, 250 mm by 4.6 mm column dimensions and security guard 4 by 30 mm phenyl-Hexyl cartridge that utilizes a liquid mobile base and soil chemical analysis were determined using Atomic Absorption Spectrophotometer, number of leaves per plant were counted directly, leaf area of each tea plant ($cm^2$) was obtained using leaf meter,

Trunk diameter was measured using a Vernier calipers,

Dry weight of *Cuscuta* spp. was measured using electronic weighing balance.

Soil pH was measured using pH (RS PRO ILDM-150H Laser Measure, 0.05 → 70m Range, ± 1.5 mm Accuracy, RS Stock No. 126-8822, Brand RS PRO) meter.

The same procedure was repeated for the control experiments.

For above ground tissue weight, each infected tea clone was cut from the soil, its moisture removed using blotting papers, and the plant dried in an oven at 80 $^oC$ for 48 hours and dry weight recorded.

For below ground tissue dry biomass each treatment had their roots uprooted, washed through a submerged 250 μm sieve with running water to remove soil particles and dried in an oven below 80 ºC and weighed using a precision scale. Percentage biomass weight of tea was determined as follows.

$$\% = \frac{W1 - W2}{W1} * 100$$

Where $W_1$ is the weight of tea before drying, $W_2$ is dry weight of Tea.

First data was collected in the initial stages of the experiment. Then after two months, and the fifth month data was collected for effects of Cuscuta infestation.

### 3.4 Statistical analyses

The data were analyzed using IBM SPSS 25 statistical software. All the tests were carried out at $p < 0.05$ significance level. There was no plant to plant interaction therefore; the differences in leaf number, leaf area, trunk diameter and total biomass from each infected clone and respective control were tested using a paired student's t-test. Differences between % chemical components in infected and control was assessed using Chi squares goodness of fit test.

### 3.5 Measurements of yield losses from *Cuscuta campestris*

Losses from *C. campestris* in the infected tea clones were assessed by comparing *C. campestris* infected tea clones with *C. campestris* free tea clones. The relative loss from *C. campestris* was calculated according to Marambe *et al*. (2002) as follows:

$$\% \, loss = \frac{(C - T)}{C} * 100$$

C-Value of the yield trait in *C. campestris* free tea clones

T-Value of the yield trait in *C. campestris* infested tea clones

The value obtained represents reduction in biological yield of tea.

### 3.6 Laboratory analyses of catechins and polyphenols

Polyphenols in tea leaves are largely linked to promotion of human health because they have high content of antioxidants (Tadesse *et al*., 2015). Caffeine in tea is a central nervous system stimulant controlling most human activities making a component of most drugs, Polyphenols and caffeine control the quality of tea.

#### 3.6.1 Leaf sampling and sampling procedure

About 600 g of 2 leaves and a bud were noted and plucked randomly from each of the infected and non-infected tea clones and separately placed in labeled khaki papers then transferred into a freezer containing ice cubes. Drying was done using a microwave for 5 minutes. This also deactivated the enzyme polyphenol oxidase from causing oxidation. Grounding was done using a coffee Miller and the powdered samples placed in Aluminium bags and stored in a dry place awaiting analysis.

#### 3.6.2 Equipment used

- Analytical electric balance: ex Adventurer OHAUS
- Agilent Technologies 1260 series with quaternary pump.
- Detector; Diode Array and multiple wavelengths Detector SL at 230nm
- Standard Auto Sampler or Preparative Auto Sampler
- Thermos tatted Column Compartment capable of maintaining a constant temperature of 40 $^{o}$C
- Computerized data acquisition system: ex Clarity Software

- Column: ZOBRAX Eclipse XBD-C18, 150mm*4.6mm, 5um

- Vacuum Degasser

- Solvent Compartment

- PTFE (hydrophilic) membrane filters, 0.45um or mini centrifuge (speed 13.0*1000rpm)

- Water purification system: ex) Milli-Q pure water system

- Sonic bath at room temperature

**3.7 Reagents**

- Acetonitrile. HPLC grade

- Methanol, HPLC grade

- Phosphoric acid 85%

- 1N hydrochloric acid

- Epigallocatechingallate (EGCg) reference standard (TFT)

- Epicatechingallate (EGg) reference standard (TFT)

- Gallocatechingallate (CGC) reference standard (TFT)

- Epigallocatechin (EGC) reference standard (TFT)

- Epicatechin (EC) reference standard (TFT)

- Catechingallate (Cg) reference standard (TFT)

- Gallocatechin (GC) reference standard (TFT)

- (+)-catechin (C) reference standard (TFT)

- Caffeine

- Gallic Acid anhydrous or monohydrate (Sigma Chemical Co.)

- Purified water from Milli-Q pure water system or equivalent

### 3.7.1 Reagent preparation

- Phosphoric acid solution
- 1 ml of phosphoric acid was diluted with purified water to make 200 ml

### 3.7.2 Mobile phase for Gradient

**Solution A-** a suitable degassed mixture of diluted phosphoric acid solution was prepared (1 in 2000) and Acetonitrile (V/V=40:1)

**Solution B-** a suitable degassed of diluted phosphoric acid solution (1 in 2000), methanol and Acetonitrile (V/V=40: 20:1) was prepared.

### 3.7.3 Chromatographic conditions

- Column: ZORBAX Eclipse XDB-C18, 150 mm *4.6 mm, 5 um
- Mobile phase: as described below
- Cycle time: 30 min
- Flow rate: 1.0 ml/min
- Injection volume: 10 UL
- Detector: 230 nm for catechin and 275 nm for caffeine
- Temperature: $40^{o}$ C
- Auto sampler temperature: $4^{o}$ C

**Table 3.3: Chromatographic conditions**

| Time (Minutes) | Solution A | Solution B | Status |
|---|---|---|---|
| 0 | 100 | 0 | Equilibrium |
| 0-3 | 100 | 0 | Isocratic |
| 3-25 | 100-0 | 0-100 | Linear gradient |
| 25-26 | 0-100 | 100-0 | Linear gradient |
| 26-30 | 0 | 0 | Isocratic |

### 3.8 Catechin stock standard preparation

10 mg of gallic as anhydrous, 40 mg of gallocatechin (-GC), 70 mg of epigallocatechin (-EGC), 30 mg of epicatechin (-EC), 40 mg of catechin (+C), 100 mg of

epigallocatechingallate (-EGCG), 20 mg of gallocatechingallate (-GCg), 30 mg of epicatechingallate (-ECg), 20 mg of catechingallate (-Cg) and 50 mg of caffeine were weighed into a 200 ml volumetric flask (Saric-Krsmanovic, 2020). The reagents were dissolved with 1ml of methanol, then water was added to make exactly 200 ml Catechin working standard preparation. 5 different concentrations standard solutions were then prepared according to Table 3.4.

**Table 3.4: Standardization table**

| Standard Name | Volume of Stock solution(ml) | Volume of 1N solution (UL) | Volume of standard solution (ml) |
|---|---|---|---|
| STD 1 | 20 | 200 | 50 |
| STD 2 | 30 | 400 | 100 |
| STD 3 | 20 | 400 | 100 |
| S TD4 | 10 | 400 | 100 |
| STD 5 | 5 | 800 | 200 |

1ml of working standard solution was dispensed into disposable plastic tube and stored below- 20 $^{o}$C. Stored working solutions was melt under ambient temperature for about 30 minutes, or until thawed. It was then mixed by shaking, and then set it in an auto sampler at 4 $^{o}$C.

### 3.9 Sample preparation (leaf tea)

- Appropriate amount of well dried tea leaf according to the proper sampling method were grinded.
- Approximately 200 mg grinded leaf was put into 100ml volumetric flask
- Addition of 40 ml of 80% methanol
- Sonication follows for 30 minutes under ambient temperature
- 1ml 1N hydrochloric acid was added and mixed well
- Addition of purified water up to approximate 90 ml
- Cooling was done in ambient temperature water bath for 30 minutes
- Top up was done with purified water to 100 ml

- Filtration of the solution by 0.45-micron PTFE filter. First 3ml should be discarded. Alternatively centrifuge the sample using a centrifuge at a speed of 13000 rpm for 10 minutes and carefully pipette the supernatant solution into HPLC vials
- Analyses of 10 micros by HPLC under the same operational condition of catechins and caffeine.

**3.10 Calculation of catechin content in tea leaf**

A working curve was made from the assay values of the standard and the catechin concentration of IGT/TE sample determined (Saric-Krsmanovic, 2020). The individual catechin content (%) W/W (as received basis) in IGT/TE was calculated using the following equation

$$\text{Individual catechin content (\%) w/w (as received basis)} = (\text{RF} * \text{Asample} * \text{vol} * \text{d})/(\text{m} * 10{,}000)$$

Where:

RF= is the response factor for the individual catechin standard component

$A_{sample}$ = is the peak area for the test sample

V = is the sample extraction volume (50ml for instant tea)

d = is the dilution factor

m = is the mass in grams, of the test sample

$$\textit{Total catechin content (\%) w/w (as received basis)} = \text{GC} + \text{EGC} + \text{Cg} + \text{C} + \text{EC} + \text{EGCg} + \text{GCg} + \text{ECG}$$

### 3.11 System suitability

The system suitability of the chromatographic system for catechin is evaluated by reproducibility, retention time, and theoretical plate count, tailing factor and resolution between the peaks.

### 3.12 Extraction of caffeine

To deactivate the enzymes, hot water at $60^0$ C was added to the grounded leaves from each tea clone and each control separately in the ratio of 1:100. The mixture was stirred thoroughly and the resulting solution evaporated using rotavapour at $60^0$ C. To the concentrate, an equal volume of dichloromethane ($CH_2Cl_2$) was added; Caffeine (chlorophyll, lipid, carbohydrates) dissolved and formed the lower layer while the polyphenols formed the top layer. The procedure was repeated 5 times. The solutions obtained were concentrated in rotavapour without vacuum (Saric-Krsmanovic, 2020).

Dichloromethane ($CH_2Cl_2$) was recovered in the matter plastered inside the round bottomed flask. The caffeine mixture was dried and filtered. Chlorophyll in the mixture was removed through addition of hot water and heated in a water bath at $80^0$ C with constant stirring for 30 minutes. The solution was evaporated and filtered to remove the suspended chlorophyll.

A few drops of hydrochloric acid were added to the filtrate to precipitate proteins which was filtered out. Calcium oxide was added to the filtrate (caffeine) to raise the pH to about 5.2. Followed by addition of charcoal. Heating was done at $80^0$ C-$90^0$ C for 5 minutes then charcoal was filtered out as residue (Saric-Krsmanovic, 2020). The filtrate was then concentrated and allowed to cool, forming a white lumpy mass, which was vacuum filtered

and dried to give a pure white caffeine crystal. Percentage change in mass was determined using the expression

$$\%C = A1 - \frac{A1 - A2}{A1} * 100$$

Where

$A_1$ is mass of the control clone.

$A_2$ is mass of infected clone.

### 3.13 Extraction of polyphenols

The undissolved remaining tea clone concentrate were mixed with ethyl acetate and 0.5g ascorbic acid (to prevent oxidation) in a separating funnel. The upper yellow layer formed was polyphenols (soluble in ethyl acetate) while the bottom layer was oil, fats and lipids (insoluble in ethyl acetate). The procedure was repeated 5 times for each tea clone treatment and control. The resulting solution was concentrated and phenols dried and stored in a desiccator to prevent stickiness (Saric-Krsmanovic, 2020).

### 3.14 HPLC analyses of polyphenols

The extracted polyphenols were placed in a 50 ml volumetric flask and HPLC grade acetone nitrite added in the ratio of 1:10. Dilution was made up to the 50 ml mark. The mixture and the standard solution were run through the HPLC test where percentage of each component was identified according to the retention time and spectrum view of the corresponding standards.

### 3.15 Soil pH procedure

Soil auger to a depth of 10 cm was used to obtain the soil samples from the treatments. A suspension was prepared by placing approximately 25 g of the gross soil in a plastic flask, 0.25 ml of distilled water was added using a graduated cylinder. Shaking of the mixture was done after every 30 minutes for 1 hour. The glass electrode was then inserted in the water -saturated soil and the pH was measured and recorded when the value was constant. Cleaning of the electrodes was done after every reading with a stream of water from a wash bottle and wiped dry with a clean tissue. A standard calibration of pH 4 and 7 was used (Saric-Krsmanovic, 2020).

### 3.16 Measurement of macro and micronutrients in soil extracts

Plant residues from the soil surface of the experimental pots were used. An auger to a depth of 10 cm was used to obtain the soil samples from the experimental pots, mixed thoroughly in a bucket and approximately 5 g sent to the laboratory. 5 g of air-dried, sieved soil was mixed with 100 mL water and placed in extraction bottle. Shaking at 200 oscillations per minute for 5 minutes on a reciprocating shaker was done. Filtration through a medium-porosity filter paper (Whatman No. 2). Analysis of the filtrate for P, K, Ca, Mg, Cu, Mn, Zn, Fe and Na was done using Atomic Absorption Spectrophotometer (AAS). Using the Mehlich 3, Morgan, and modified Morgan soil extracts.

Phosphorus was measured by the resin method; 2.5 g of ground soil was passed through a 2.0 mm sieve into a polystyryne flask. 25 ml of distilled water was added and a glass marble (1.8 cm diameter). The flask was closed and shaken for 15 mins on a rotary motion shaker at 220 rpm followed by the removal of the glass marbles. With the help of a water stream, the suspension was then transferred to a 0.4mm opening polyester netting sieve for the

separation of resin from soil. The resin was then rinsed with a minimum of water to remove the finer soil particles, until the washing was clear. The resin with the soil was transferred to the flask using a measured volume of 50 ml solution of 1M NaCl in 0.1M HCl for 30 minutes to remove carbon dioxide formed. The flask was closed and shaking done for 1-hour phosphorus was extracted filtered. The standard and the samples were kept in the dark for colour development (Saric-Krsmanovic, 2020).

The standard and sample absorbance were then measured at 400 nm wavelength in a suitable calorimeter (Bosch GLM 30 Laser Measure, 0.15 → 30m Range, ±2 mm Accuracy) starting with the blank, standards and then the samples. The concentrations of phosphorus in parts per million (ppm) was read off from the calibration curve of absorbance against the concentrations of the standard series; For potassium 10 g of the dry soil was placed in 100 ml plastic bottles, 50 ml of 1M ammonium nitrate solution was added shaken for 30minutes and filtered into 50 Ml test tubes. Of these, 1ml was diluted with 5 ml of 0.5% strontium chloride solution. The flame photometer was then used to measure the concentration of potassium in the soil in parts per million (ppm).

The same procedure was repeated to obtain the sample solutions for calcium with the standard calcium of the flame photometer at wavelength 422.7 nm. The concentration of calcium in the soil samples was expressed in ppm. Magnesium and Manganese were analyzed using an atomic absorption spectrometer their standard series and solution samples were sprayed into the flame atomic absorption spectrometer at a wavelength 285.2 nm. Their absorbance was measured against concentration of the standard series and read off the concentration of the sample. The concentrations of magnesium and manganese in the soil sample expressed in ppm (Saric-Krsmanovic, 2020).

For Zinc, copper and iron their solutions were sprayed starting the blank. The standard series and sample solutions were sprayed into the flame atomic absorption spectrophotometric at a wavelength of 213.9 nm, 324.8 nm and 248.3 nm respectively. Their absorbance in the standard series were measured, a calibration curve of absorbance against concentration drawn and based on the curves the concentrations were read off and expressed in parts per million (ppm). Flame photometry was used to determine the concentration of sodium in the soil samples. Distilled water was used as blank and the peak reading was set according to the instructions using the concentrated sodium chloride solution (100 mg/ml).

The emissions of the standard solution and those of the samples were measured a working curve plotted and concentration of sodium was determined from the curve in Mm. The results from the various techniques used to measure the micronutrients in the soil extracts should, in general, be comparable although P measured by ICP may be higher than when measured calorimetrically for many soils. Because of this difference, soil test calibrations were based on the specific analytical method routinely used. Because of the greater efficiency of ICPs, the majority of laboratories utilize this approach when measuring the macro and micronutrients in soil extracts although several utilize a colorimetric method for phosphorus.

**3.18 Data analysis**

Data was analyzed in SPSS version 20 for mean differences. t test was used to determine the differences in mean between infected and control. Chi square was used to determine significant difference between observed ad expected frequencies at 0.05 significant difference.

# CHAPTER FOUR

# RESULTS

## 4.1 Effects of dodder on Tea growth parameters

There were several variables that were assessed to determine the effect of *Cuscuta* on tea growth parameters. These were; Trunk diameter, Leaf area ($cm^2$), Number of leaves, Biomass of below ground tissue, Biomass of above ground tissue and Effect of *Cuscuta campestris* on two leaves and a bud (harvest-biomass) (g).

### 4.1.1 Trunk diameter

There were six clones tested for differences in shrub diameter between infected and control conditions. Among the six clones tested, only TRFK 306/1 had a significance difference (t=3.59, p=0.02) between infected (1.04±0.15) and control (0.7±0.04) (Table 4.1).

Table 4.1: Mean difference in trunk diameter between infected and control tea clones conditions

| | Mean | | | |
|---|---|---|---|---|
| **Clone** | **Infected** | **Control** | **t test** | **p value** |
| TRFK430/90 | 0.91±0.09 | 0.92±0.03 | -0.2198 | 0.8367 |
| TRFK31/8 | 1.18±0.19 | 1.00±0.08 | 1.4154 | 0.2298 |
| TRFK306/1 | 1.04±0.15 | 0.7±0.04 | 3.5969 | 0.0228* |
| TRFK303/577 | 1.07±0.03 | 0.97±0.18 | 0.8857 | 0.4258 |
| TRFK301/4 | 1.07±0.16 | 1.10±0.28 | -0.1403 | 0.8951 |
| EPKTN14-3 | 1.03±0.17 | 1.09±0.08 | -0.5312 | 0.6233 |

*Significant mean differences are indicated with **
*TRFK- Tea Research Foundation Kericho*

### 4.1.2 Leaf area ($cm^2$)

For the leaf area, one clone had a significance differences between means of infected and control. Mean leaf area for the infected TRFK306/1 clone (40.33±6.50) was significantly different (t=-3.01, p=0.03) from its mean for the control (46.00±14.00) (Table 4.2)

Table 4.2: Mean difference in Leaf area ($cm^2$) between infected and control tea clones

| | Mean | | | |
|---|---|---|---|---|
| **Clone** | **Infected** | **Control** | **t- test** | **p value** |
| TRFK430/90 | 30.33±1.52 | 37.00±4.35 | -2.5000 | 0.0667 |
| TRFK31/8. | 40.33±6.50 | 47.33±7.57 | -1.2144 | 0.2913 |
| TRFK306/1. | 21.33±2.30 | 46.00±14.00 | -3.0110 | 0.0395* |
| TRFK303/577. | 33.00±3.00 | 38.00±2.00 | -2.4019 | 0.0742 |
| TRFK301/4. | 29.33±2.08 | 34.33±2.08 | -2.9417 | 0.0453 |
| EPKTN14-3. | 37.66±5.85 | 38.66±6.42 | -0.1991 | 0.8518 |

*Significant mean differences are indicated with **
*TRFK- Tea Research Foundation Kericho*

### 4.1.3 Number of leaves in treatment and control infected and controls experiments

There was no significance differences in means of number of infected leaves and controls for all the six clones. Mean number of infected leaves of TRFK303/577 clone was highest (73.00±5.29) followed by EPKTN14-3 (61.66±4.72) with insignificant differences from mean control (Table 4.3

Table 4.3: Mean differences in number of leaves in treatment and control experiments

| | Mean | | | |
|---|---|---|---|---|
| **Clone** | **Infected** | **Control** | **t- test** | **p value** |
| TRFK430/90 | 24.33±8.73 | 31.66±12.09 | -0.8512 | 0.4426 |
| TRFK31/8. | 30.66±10.40 | 37.33±15.69 | -0.6131 | 0.5729 |
| TRFK306/1. | 39.33±17.21 | 47.33±2.08 | -0.7991 | 0.4689 |
| TRFK303/57 | 73.00±5.29 | 75.00±5.00 | -0.4758 | 0.6590 |
| TRFK301/4. | 33.33±14.57 | 43.33±11.93 | -0.9197 | 0.4097 |
| EPKTN14-3. | 61.66±4.72 | 7.00±8.96 | -2.1652 | 0.0963 |

*Significant mean differences are indicated with **
*TRFK- Tea Research Foundation Kericho*

### 4.1.4 Biomass of below ground tissue in g for treatment and control experiment

Differences in mean control and mean infected of root biomass in g was assessed for all the six clones. All the six clones showed a significant difference. For the TRFK430/90 clone, mean biomass of the infected root (85.63±0.55g) was significantly different (t=12.92, p=0.0001) from mean biomass for controls (79.66±0.57g) (Table 4.4).

Table 4.4: Below ground tissue biomass (g)

| | Mean | | | |
|---|---|---|---|---|
| **Clone** | **Infected** | **Control** | **t- test** | **p value** |
| TRFK430/90. | 85.63±0.55 | 79.66±0.57 | 12.9200 | 0.0001* |
| TRFK31/8. | 47.63±0.55 | 31.43±0.57 | 37.2700 | 0.0000* |
| TRFK306/1. | 37.20±1.05 | 31.8±0.10 | 8.7900 | 0.0001* |
| TRFK303/577. | 122.63±0.55 | 125.0±1.00 | -3.5900 | 0.0229* |
| TRFK301/4. | 78.2±0.10 | 50.66±0.66 | 70.8200 | 0.0000* |
| EPKTN14-3. | 47.63±0.55 | 31.43±0.51 | 37.2700 | 0.0000* |

*Significant mean differences are indicated with **
*TRFK- Tea Research Foundation Kericho*

### 4.1.5 Biomass of above ground tissue in infected and control clones

There was no significant difference in means of biomass of above ground tissue in infected and controls of above ground tissue TRFK430/90 clone. Mean number of control biomass of TRFK306/1 clone was highest (125.00±1.00) with significant differences from mean control (Table4.5).

Table 4.5: Biomass of above ground tissue in infected and control clones

| | Mean | | | |
|---|---|---|---|---|
| **Clone** | **Infected** | **Control** | **t-test** | **p value** |
| TRFK430/90. | 81.6±7.73 | 68.76±6.62 | 2.1830 | 0.0944 |
| TRFK31/8. | 78.20±0.10 | 50.66±0.66 | 70.8289 | 0.0003* |
| TRFK306/1. | 122.63±0.55 | 125.00±1.00 | -3.5906 | 0.0229* |
| TRFK303/577. | 85.63±0.55 | 79.66±0.57 | 12.9500 | 0.0002* |
| TRFK301/4. | 47.63±0.55 | 31.43±0.51 | 37.2700 | 0.0000* |
| EPKTN14-3. | 94.30±2.98 | 80.13±2.62 | 6.71900 | 0.0025* |

*Significant mean differences are indicated with **
*TRFK- Tea Research Foundation Kericho*

### 4.1.6 Effect of *Cuscuta campestris* on two leaves and a bud (harvest biomass in g)

Differences in mean control and mean infected of tea harvest biomass in g were assessed for all the six clones. All the six clones showed a significant difference. For the TRFK430/90 clone, mean biomass of the infected (2.53±0.55 g) was significantly different (t=-3.47, p=0.02) from mean biomass control (4.15±0.58 g). Clone TRFK31/8 showed significance difference (t=-10.99, p=0.00) between the biomass means of infected (3.91±0.50 g) and control (8.76±0.57 g). For the TRFK306/1 clone, there was a significant difference (t=-4.18, p=0.01), between means of infected biomass (2.67±0.61g) and control (4.17±0.06g) (Table 4.6).

Table 4.6. Mean difference in two leaves and a bud (harvest -biomass (g) in treatment and control experiments

| | | Mean | | |
|---|---|---|---|---|
| **Clone** | **Infected** | **Control** | **t-test** | **p value** |
| TRFK430/90. | 2.53±0.55 | 4.15±0.58 | -3.4728 | 0.0255* |
| TRFK31/8. | 3.91±0.50 | 8.76±0.57 | -10.9922 | 0.0003* |
| TRFK306/1. | 2.67±0.61 | 4.17±0.06 | -4.18167 | 0.0138* |
| TRFK303/577. | 4.01±0.10 | 6.62±0.28 | -15.1298 | 0.0001* |
| TRFK301/4. | 4.81±0.27 | 10.87±0.57 | -16.4655 | 0.0000* |
| EPKTN14-3. | 3.06±0.91 | 6.45±0.61 | -5.31153 | 0.0060* |

*Significant mean differences are indicated with **
*TRFK- Tea Research Foundation Kericho*

## 4.2 Effects of *Cuscuta campestris* on tea quality

### 4.2.1 Mean difference in %Gallate (GA) between controls and infected

The mean % gallate for control for clones TRFK31/8, TRFK303/577, EPKTN 14/3 and TRFK430/90, were 0.00% while for clones TRFK306/1 and TRFK301/4 were 0.07% and 0.08% respectively. For the infected, the mean % gallate was 0.06 for TRFK31/8 while all

the other clones had a mean of 0.00%. There was no significant difference between control and infected mean % gallate for clones TRFK303/577, EPKTN 14/3 and TRFK430/90.

Table 4.7: Mean difference in %Gallate (GA) between controls and infected

| Clones | % mean | Catechins | Chi square test ($\chi^2$) | df | p value |
|---|---|---|---|---|---|
| TRFK31/8 | Control | 0.00 | - | - | - |
| | Infected | 0.06 | | | |
| TRFK303/577 | Control | 0.00 | - | - | - |
| | infected | 0.00 | | | |
| TRFKTN 14/3 | Control | 0.00 | - | - | - |
| | Infected | 0.00 | | | |
| TRFK430/90 | Control | 0.00 | - | - | - |
| | Infected | 0.00 | | | |
| TRFK306/1 | Control | 0.07 | - | - | - |
| | Infected | 0.00 | | | |
| TRFK301/4 | Control | 0.07 | - | - | - |
| | Infected | 0.00 | | | |

### 4.2.2 Mean differences in % gallocatechin (GC) for Catechins between control and infected clones

The mean % gallocatechin for control was highest (0.4%) for clones TRFK31/8, while clones TRFK306/1 had the lowest (0.04%). For the infected clones, the mean % gallocatechin was highest in clone TRFK430/900 and lowest in TRFK306/1. There was no significant difference between control and infected mean % gallocatechin for all Catechins (Table 4.7).

Table 4.8: Mean differences in % gallocatechin between control and infected clones

| Clones | % mean | Catechins | Chi square test ($\chi^2$) | df | p value |
|---|---|---|---|---|---|
| TRFK31/8 | Control | 0.48 | 0.9392 | 1 | 0.3325 |
| | Infected | 0.59 | | | |
| TRFK303/577 | Control | 0.30 | 0.7400 | 1 | 0.3897 |
| | infected | 0.44 | | | |
| TRFKTN 14/3 | Control | 0.36 | 0.6500 | 1 | 0.4201 |
| | Infected | 0.29 | | | |
| TRFK430/90 | Control | 0.36 | 1.1078 | 1 | 0.2926 |
| | Infected | 0.55 | | | |
| TRFK306/1 | Control | 0.04 | 0.0900 | 1 | 0.7642 |
| | Infected | 0.05 | | | |
| TRFK301/4 | Control | 0.25 | 0.3700 | 1 | 0.5430 |
| | Infected | 0.12 | | | |

*Significant mean differences are indicated with **
*TRFK- Tea Research Foundation Kericho*

### 4.2.3 Mean differences in % epigallocatechin (EGC) between control and infected clones

The mean % epigallocatechin for control was highest (5.57%) for clones TRFK31/8, while clones TRFK306/1 had the lowest (1.00%). For the infected clones, the mean % epigallocatechin was highest in clone TRFK430/900 and lowest in TRFK306/1. There was no significant difference between control and infected mean % epigallocatechin for all catechins (Table 4.8).

**Table 4.9. Mean differences in % epigallocatechin between control and infected clones**

| Clones | test | Mean | Chi square test ($\chi^2$) | df | p value |
|---|---|---|---|---|---|
| TRFK31/8 | Control | 4.20 | 0.117605 | 1 | 0.7316 |
| | Infected | 4.61 | | | |
| TRFK303/577 | Control | 3.36 | 0.497337 | 1 | 0.4807 |
| | Infected | 5.09 | | | |
| EPKTN 14/3 | Control | 4.64 | 0.0105 | 1 | 0.9181 |
| | Infected | 5.04 | | | |
| TRFK430/90 | Control | 5.57 | 0.0781 | 1 | 0.7798 |
| | Infected | 5.50 | | | |
| TRFK306/1 | Control | 1.00 | 0.3660 | 1 | 0.5451 |
| | Infected | 1.82 | | | |
| TRFK301/4 | Control | 1.81 | 0.1137 | 1 | 0.7359 |
| | Infected | 1.57 | | | |

*Significant mean differences are indicated with **
*TRFK- Tea Research Foundation Kericho*

### 4.2.4 Mean differences in % caffeine (+C) between control and infected clones

Mean % caffein were high in clone TRFK301/4 control and low in TRFK306/1. There was no significant difference in % caffein means between control and infected (Table 4.9).

Table 4.10: Mean differences in % caffeine between control and infected clones

| Clones | Test | Mean | Chi square test ($\chi^2$) | df | p value |
|---|---|---|---|---|---|
| TRFK31/8 | Control | 0.15 | 0.3000 | 1 | 0.5839 |
| | Infected | 0.15 | | | |
| TRFK303/577 | Control | 0.28 | 0.4100 | 1 | 0.5220 |
| | Infected | 0.13 | | | |
| EPKTN 14/3 | Control | 0.20 | 0.3700 | 1 | 0.5430 |
| | Infected | 0.17 | | | |
| TRFK430/90 | Control | 0.17 | 0.3600 | 1 | 0.5485 |
| | Infected | 0.19 | | | |
| TRFK306/1 | Control | 0.07 | 0.1100 | 1 | 0.7401 |
| | Infected | 0.04 | | | |
| TRFK301/4 | Control | 0.35 | 0.5800 | 1 | 0.4463 |
| | Infected | 0.23 | | | |

*Significant mean differences are indicated with **
*TRFK- Tea Research Foundation Kericho*

### 4.2.5 Mean differences in % epigallocatechingallate (Egg) between control and infected clones

Catechins with the highest mean % epigallocatechingallate was TRFK 31/8 (control) followed by TRFK430/90 and EPKTN 14/3 with no significant difference with the infected as portrayed in table 4.11. Clone (control) with lowest mean % epigallocatechingallate were again not significantly different from the lowest infected.

Table 4.11: Mean differences in % epigallocatechingallate between control and infected clones.

| Clones | Test | % Mean | Chi square test ($\chi^2$) | df | p value |
|---|---|---|---|---|---|
| TRFK31/8 | Control | 10.34 | 0.0000 | 1 | 0.9487 |
| | Infected | 9.95 | | | |
| TRFK303/577 | Control | 6.54 | 0.3300 | 1 | 0.5618 |
| | Infected | 5.37 | | | |
| TRFKTN 14/3 | Control | 9.38 | 0.0000 | 1 | 0.9868 |
| | Infected | 8.55 | | | |
| TRFK430/90 | Control | 9.28 | 0.0700 | 1 | 0.7774 |
| | Infected | 8.36 | | | |
| TRFK306/1 | Control | 3.48 | 0.0000 | 1 | 0.9518 |
| | Infected | 2.67 | | | |
| TRFK301/4 | Control | 5.10 | 0.5300 | 1 | 0.4642 |
| | Infected | 2.57 | | | |

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*TRFK- Tea Research Foundation Kericho*

### 4.2.6 Mean differences in % epicatechi (EC) between control and infected clones

Infected TRFK301/4 catechins had high % epicatechin mean of TRFK3.60 followed by the control recording a % epicatechin mean of 3.00. For clone TRFK31/8, TRFK306/1 and TRFK301/4, the mean % epicatechin was high in infected than in control. There was no significance difference between control and infected % epicatechin means (Table 4.12).

Table 4.12: Mean differences in % Epicatechin (EC) between control and infected clones

| Clones | Test | % Mean | Chi square test ($\chi^2$) | df | p value |
|---|---|---|---|---|---|
| TRFK38/8 | Control | 0.88 | 0.0157 | 1 | 0.9000 |
| | Infected | 0.95 | | | |
| TRFK303/577 | Control | 1.95 | 0.3279 | 1 | 0.5669 |
| | Infected | 1.11 | | | |
| EPKTN 14/3 | Control | 1.70 | 0.3333 | 1 | 0.5637 |
| | Infected | 1.30 | | | |
| TRFK430/90 | Control | 1.35 | 0.1073 | 1 | 0.7432 |
| | Infected | 1.17 | | | |
| TRFK306/1 | Control | 0.83 | 0.0609 | 1 | 0.8050 |
| | Infected | 0.85 | | | |
| TRFK301/4 | Control | 3.00 | 0.1757 | 1 | 0.6750 |
| | Infected | 3.60 | | | |

*Significant mean differences are indicated with **
*TRFK- Tea Research Foundation Kericho*

### 4.2.7 Mean differences in % gallocatechingallate (GCg) between control and infected clones

EPKTN 14/3 and TRFK38/8 clone had high mean % gallocatechingallate that in control while the rest had high mean % gallocatechingallate in control than in infected. There was no significance difference between control and infected % gallocatechingallate means (Table 4.13).

Table 4.13: Mean differences in % galllocatechingallate between control and infected clones

| Clones | Test | % Mean | Chi square test ($\chi^2$) | df | pvalue |
|---|---|---|---|---|---|
| TRFK31/8 | Control | 0.19 | 0.5900 | 1 | 0.4424 |
| | Infected | 0.40 | | | |
| TRFK303/577 | Control | 0.14 | 0.2400 | 1 | 0.6242 |
| | Infected | 0.10 | | | |
| TEPKN 14/3 | Control | 0.25 | 0.6000 | 1 | 0.4386 |
| | Infected | 0.35 | | | |
| TRFK430/90 | Control | 0.37 | 0.5100 | 1 | 0.4751 |
| | Infected | 0.14 | | | |
| TRFK306/1 | Control | 0.24 | 0.2800 | 1 | 0.5967 |
| | Infected | 0.04 | | | |
| TRFK301/4 | Control | 0.29 | 0.3800 | 1 | 0.5376 |
| | Infected | 0.09 | | | |

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*TRFK- Tea Research Foundation Kericho*

### 4.2.8 Mean differences in %epicatechingallate (ECg) between control and infected clones

Clone TRFK301/4 had the highest mean % epicatechingallate in control (4.94 %.) In all Clone, mean % epicatechingallate was high in all controls than in infected with no significant difference (Table 4.14).

Table 4.14: Mean differences in % ECg between control and infected clones

| Clones | Test | % Mean | Chi square goodness of fit test ($\chi^2$) | df | p value |
|---|---|---|---|---|---|
| TRFK31/8 | Control | 1.74 | 0.1219 | 1 | 0.7270 |
| | Infected | 1.62 | | | |
| TRFK303/577 | Control | 2.58 | 1.1244 | 1 | 0.2890 |
| | Infected | 1.08 | | | |
| EPKTN 14/3 | Control | 2.66 | 0.3023 | 1 | 0.5824 |
| | Infected | 1.76 | | | |
| TRFK430/90 | Control | 1.86 | 0.0876 | 1 | 0.7671 |
| | Infected | 1.59 | | | |
| TRFK306/1 | Control | 2.40 | 0.3398 | 1 | 0.5599 |
| | Infected | 0.99 | | | |
| TRFK301/4 | Control | 4.93 | 0.5094 | 1 | 0.4754 |
| | Infected | 2.95 | | | |

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*TRFK- Tea Research Foundation Kericho*

### 4.2.9 Mean differences in % catechingallate (Cg) between control and infected clones

Clone TRFK303/577 had no % catechingallate in both control and infected. For the Clone TRFK430/90, EPKTN 14/3 and TRFK303/577 the mean % catechingallate was high in infected than in control with no significant difference (Table 4.15).

Table 4.15: Mean differences in % catechingallate between control and infected clones

| Clones | Test | % Mean | Chi square test ($\chi^2$) | df | p value |
|---|---|---|---|---|---|
| TRFK31/8 | Control | 0.06 | 0.1300 | 1 | 0.7184 |
| | Infected | 0.07 | | | |
| TRFK303/577 | Control | 0.00 | - | 1 | - |
| | Infected | 0.00 | | | |
| EPKTN 14/3 | Control | 0.05 | 0.1100 | 1 | 0.7401 |
| | Infected | 0.06 | | | |
| TRFK430/90 | Control | 0.10 | 0.2400 | 1 | 0.6242 |
| | Infected | 0.14 | | | |
| TRFK306/1 | Control | 0.29 | 0.4100 | 1 | 0.5220 |
| | Infected | 0.12 | | | |
| TRFK301/4 | Control | 0.05 | 0.0500 | 1 | 0.8231 |
| | Infected | 0.00 | | | |

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### 4.2.10 Mean differences in %Total (8) Catechins between control and infected clones

Mean % total (8) Catechins was high for TRFK303/577, EPKTN 14/3, TRFK430/90, TRFK306/1 and TRFK301/4 (Table 4.16).

Table 4.16: Mean differences in % total (8) catechins between control and infected clones

| Clones | Test | % Mean | Chi square test ($\chi^2$) | df | p value |
|---|---|---|---|---|---|
| 31TRFK/8 | Control | 18.04 | 0.0039 | 1 | 0.9498 |
| | Infected | 18.34 | | | |
| TRFK303/577 | Control | 15.14 | 0.1477 | 1 | 0.7007 |
| | Infected | 13.31 | | | |
| EPKTN 14/3 | Control | 19.23 | 0.0290 | 1 | 0.8646 |
| | Infected | 17.51 | | | |
| TRFK430/90 | Control | 19.05 | 0.0300 | 1 | 0.8624 |
| | Infected | 17.63 | | | |
| TRFK306/1 | Control | 8.32 | 0.0677 | 1 | 0.7946 |
| | Infected | 6.58 | | | |
| TRFK301/4 | Control | 15.77 | 0.9305 | 1 | 0.3347 |
| | Infected | 11.11 | | | |

*Significant mean differences are indicated with **
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### 4.2.11 Mean differences in % total gallatecatechins between control and infected clones

Mean % total gallatecatechins was high in all controls than in infected with no significant difference (Table 4.17).

Table 4.17: Mean differences in % total gallatecatechins between control and infected clones

| Clones | Test | % Mean | Chi square test ($\chi^2$) | df | p value |
|---|---|---|---|---|---|
| TRFK31/8 | Control | 12.33 | 0.0050 | 1 | 0.9403 |
| | Infected | 12.04 | | | |
| TRFK303/577 | Control | 9.26 | 0.2552 | 1 | 0.6134 |
| | Infected | 6.55 | | | |
| EPKTN 14/3 | Control | 12.33 | 0.0434 | 1 | 0.8348 |
| | Infected | 10.72 | | | |
| TRFK430/90 | Control | 11.61 | 0.1843 | 1 | 0.6677 |
| | Infected | 10.23 | | | |
| TRFK306/1 | Control | 6.39 | 0.3960 | 1 | 0.5291 |
| | Infected | 3.82 | | | |
| TRFK301/4 | Control | 10.36 | 1.0026 | 1 | 0.3167 |
| | Infected | 5.60 | | | |

*Significant mean differences are indicated with **
*TRFK- Tea Research Foundation Kericho*

### 4.2.12 Mean differences in Gallate Ratio (%) Catechins between control and infected clones

All the control Clones (TRFK31/8, TRFK303/577, EPKTN 14/3, TRFK430/90, TRFK306/1 and TRFK301/4) had high levels of mean Gallate Ratio (%) Clone as compared with those infected even though the difference was not significant (Table 4.18).

Table 4.18: Mean differences in Gallate ratio (%) catechins between control and infected clones

| Clones | Test | % Mean | Chi square test ($\chi^2$) | df | p value |
|---|---|---|---|---|---|
| TRFK31/8 | Control | 68.34 | 0.0298 | 1 | 0.86 |
| | Infected | 65.60 | | | |
| TRFK303/577 | Control | 61.17 | 1.3059 | 1 | 0.2531 |
| | Infected | 49.19 | | | |
| EPKTN 14/3 | Control | 64.14 | 0.0726 | 1 | 0.7875 |
| | Infected | 61.19 | | | |
| TRFK430/90 | Control | 60.92 | 0.0757 | 1 | 0.7832 |
| | Infected | 58.00 | | | |
| TRFK306/1 | Control | 76.78 | 2.6781 | 1 | 0.1017 |
| | Infected | 58.03 | | | |
| TRFK301/4 | Control | 65.69 | 2.2056 | 1 | 0.1375 |
| | Infected | 50.38 | | | |

*Significant mean differences are indicated with **
*TRFK- Tea Research Foundation Kericho*

### 4.2.13 Mean differences in % Caffeine Catechins between control and infected clones

Mean % caffeine catechins was high in control than in infected clones for all clones with no significant difference (Table 4.19).

Table 4.19: Mean differences in % caffeine catechins between control and infected clones

| Clones | Test | % Mean | Chi square test ($\chi^2$) | df | p value |
|---|---|---|---|---|---|
| TRFK31/8 | Control | 3.92 | 0.1506 | 1 | 0.6980 |
| | Infected | 3.40 | | | |
| TRFK303/577 | Control | 2.64 | 0.2895 | 1 | 0.5905 |
| | Infected | 1.82 | | | |
| EPKTN 14/3 | Control | 3.14 | 0.0010 | 1 | 0.9741 |
| | Infected | 2.94 | | | |
| TRFK430/90 | Control | 2.99 | 0.0216 | 1 | 0.8829 |
| | Infected | 2.66 | | | |
| TRFK306/1 | Control | 1.93 | 0.0684 | 1 | 0.7937 |
| | Infected | 1.58 | | | |
| TRFK301/4 | Control | 2.65 | 0.3385 | 1 | 0.5607 |
| | Infected | 1.67 | | | |

*Significant mean differences are indicated with **
*TRFK- Tea Research Foundation Kericho*

### 4.2.14 Mean differences in catechins caffein /catechins ratio (%) between control and infected clones

Mean catechins caff. /cat. ratio (%) was high in controls of TRFK31/8, TRFK303/577, TRFK430/90 and TRFK301/4 but low in EPKTN 14/3 and TRFK306/1 when compared with the infected. There was no significant difference between Mean catechins caff. /cat. ratio (%) controls and infected (Table 4.20).

Table 4.20: Mean differences in catechins caff. / cat. ratio (%) between control and infected clones

| Clones | Test | % Mean | Chi square test ($\chi^2$) | df | p value |
|---|---|---|---|---|---|
| TRFK31/8 | Control | 21.70 | 0.2380 | 1 | 0.6256 |
| | Infected | 18.54 | | | |
| TRFK303/577 | Control | 17.44 | 0.2896 | 1 | 0.5904 |
| | Infected | 13.67 | | | |
| EPKTN 14/3 | Control | 16.33 | 0.0304 | 1 | 0.8614 |
| | Infected | 16.76 | | | |
| TRFK430/90 | Control | 15.69 | 0.0345 | 1 | 0.8525 |
| | Infected | 15.06 | | | |
| TRFK306/1 | Control | 23.19 | 0.0221 | 1 | 0.8818 |
| | Infected | 24.02 | | | |
| TRFK301/4 | Control | 16.81 | 0.1272 | 1 | 0.7213 |
| | Infected | 14.98 | | | |

*Significant mean differences are indicated with **
*TRFK- Tea Research Foundation Kericho*

### 4.4 Effects of *Cuscuta campestris* on uptake of nutrients by tea clones

#### 4.4.1 Soil pH levels

Differences in mean control and mean normal pH was assessed for all the six clones. All the six clones showed no significant difference between the normal and the control (Table 4.21).

Table 4.21: Soil pH levels

| | Mean | | | |
|---|---|---|---|---|
| **Clones** | **Infected** | **Control** | **t-test** | **p value** |
| TRFK430/90 | 4.23±0.55 | 4.20±0.57 | 6.9200 | 0.837 |
| TRFK31/8 | 3.84±0.55 | 3.92±0.01 | 7.2700 | 0.2298 |
| TRFK306/1 | 3.86±1.05 | 3.92±0.10 | 8.7900 | 0.2228 |
| TRFK303/577 | 4.12±0.55 | 4.31±1.00 | 3.5900 | 0.4258 |
| TRFK301/4 | 3.88±0.10 | 4.22±0.66 | 7.8200 | 0.8951 |
| EPKTN14-3 | 4.04±0.55 | 4.28±0.51 | 3.2700 | 0.6233 |

*Significant mean differences are indicated with **
*TRFK- Tea Research Foundation Kericho*

#### 4.4.2 Soil potassium (K) levels (mg)

Differences in mean control and mean normal were assessed for all the six clones. All the six clones showed no significant difference between the normal and the control (Table 4.22).

**Table 4.22: Soil potassium (K) levels**

| | Mean | | | |
|---|---|---|---|---|
| **Clones** | **Infected** | **Control** | **t-test** | **p value** |
| TRFK430/90 | 201±0.55 | 170±0.57 | 3.2700 | 0.8950 |
| TRFK31/8 | 196±0.55 | 162±0.01 | 7.2700 | 0.6230 |
| TRFK306/1 | 200±1.05 | 172±0.10 | 3.5900 | 0.6230 |
| TRFK303/577 | 681±0.55 | 194±1.00 | 8.7900 | 0.8910 |
| TRFK301/4 | 296±0.10 | 286±0.66 | 7.8200 | 0.6240 |
| EPKTN14-3 | 200±0.55 | 186±0.51 | 6.9200 | 0.6230 |

*Significant mean differences are indicated with **
*TRFK- Tea Research Foundation Kericho*

### 4.4.3 Soil calcium (Ca) levels (mg)

Differences in mean control and mean infected calcium was assessed for all the six clones. All the six clones showed no significant difference between the normal and the control (Table 4.23).

Table 4.23: Soil calcium levels

| | Mean | | | |
|---|---|---|---|---|
| **Clones** | **Infected** | **Control** | **t- test** | **p value** |
| TRFK430/90 | 74.01±0.55 | 74.11±0.57 | 8.7900 | 0.6230 |
| TRFK31/8 | 85.21±0.55 | 86.1±0.01 | 3.2700 | 0.6230 |
| TRFK306/1 | 116.22±1.05 | 116.50±0.10 | 3.5900 | 0.8950 |
| TRFK303/577 | 58.12±0.55 | 57.21±1.00 | 4.1200 | 0.8910 |
| TRFK301/4 | 48.21±0.10 | 48.21±0.66 | 3.2200 | 0.6240 |
| EPKTN14-3 | 116.32±0.55 | 116.17±0.51 | 7.2700 | 0.6230 |

*Significant mean differences are indicated with **
*TRFK- Tea Research Foundation Kericho*

### 4.4.4 Soil magnesium (Mg) levels (mg)

Differences in mean control and mean infected magnesium were assessed for all the six clones. All the six clones showed no significant difference between the normal and the control (Table 4.24)

Table 4.24: Soil magnesium levels

| | Mean | | | |
|---|---|---|---|---|
| **Clones** | **Infected** | **Control** | **t-test** | **p value** |
| TRFK430/90 | 22.01±0.55 | 21.11±0.57 | 4.2700 | 0.8950 |
| TRFK31/8 | 24.21±0.55 | 20.1±0.01 | 7.2700 | 0.4200 |
| TRFK306/1 | 38.22±1.05 | 17.50±0.10 | 6.5900 | 0.8150 |
| TRFK303/577 | 18.12±0.55 | 20.21±1.00 | 8.7900 | 0.8910 |
| TRFK301/4 | 16.21±0.10 | 18.21±0.66 | 4.8200 | 0.6240 |
| EPKTN14-3 | 38.32±0.55 | 30.17±0.51 | 6.3200 | 0.6230 |

*Significant mean differences are indicated with **
*TRFK- Tea Research Foundation Kericho*

### 4.4.5 Soil phosphorus (P) levels (mg)

Differences in mean control and mean infected phosphorus was assessed for all the six clones. All the six clones showed no significant difference between the normal and the control.

Table 4.25: Soil phosphorus levels

| | Mean | | | |
|---|---|---|---|---|
| **Clones** | **Infected** | **Control** | **t- test** | **p value** |
| TRFK430/90 | 34.01±0.55 | 38.11±0.57 | 43.2700 | 0.6670 |
| TRFK31/8 | 37.21±0.55 | 36.1±0.01 | 1.2700 | 0.2910 |
| TRFK306/1 | 25.22±1.05 | 23.50±0.10 | 4.5900 | 0.3950 |
| TRFK303/577 | 30.12±0.55 | 31.21±1.00 | 6.7900 | 0.7420 |
| TRFK301/4 | 34.21±0.10 | 33.21±0.66 | 5.8200 | 0.4230 |
| EPKTN14-3 | 25.32±0.55 | 26.17±0.51 | 2.9200 | 0.851 |

*Significant mean differences are indicated with **
*TRFK- Tea Research Foundation Kericho*

### 4.4.6 Soil manganese (Mn) levels (mg)

Differences in mean control and mean infected manganese was assessed for all the six clones. All the six clones showed no significant difference between the normal and the control (Table 4.26).

Table 4.26: Soil manganese levels

| | Mean | | | |
|---|---|---|---|---|
| **Clones** | **Infected** | **Control** | **t-test** | **p value** |
| TRFK430/90 | 66.01±0.55 | 61.11±0.57 | 6.7900 | 0.6570 |
| TRFK31/8 | 63.21±0.55 | 61.1±0.01 | 5.8200 | 0.3910 |
| TRFK306/1 | 159.22±1.05 | 153.50±0.10 | 4.5900 | 0.3850 |
| TRFK303/577 | 77.12±0.55 | 76.21±1.00 | 1.3400 | 0.4420 |
| TRFK301/4 | 72.21±0.10 | 70.21±0.66 | 4.2000 | 0.4260 |
| EPKTN14-3 | 159.32±0.55 | 156.17±0.51 | 2.9200 | 0.8310 |

*Significant mean differences are indicated with **
*TRFK- Tea Research Foundation Kericho*

### 4.4.7 Soil zinc (Zn) levels (mg)

Differences in mean control and mean infected zinc were assessed for all the six clones. All the six clones showed no significant difference between the normal and the control (Table 4.27).

Table 4.27: Soil zinc levels

| | Mean | | | |
|---|---|---|---|---|
| **Clones** | **Infected** | **Control** | **t-test** | **p value** |
| TRFK430/90 | 19.01±0.55 | 21.11±0.57 | 4.7900 | 0.2570 |
| TRFK31/8 | 20.21±0.55 | 18.1±0.01 | 5.8200 | 0.3910 |
| 306/1 | 21.22±1.05 | 19.50±0.10 | 4.1900 | 0.1850 |
| TRFK303/577 | 19.12±0.55 | 21.21±1.00 | 2.3400 | 0.5420 |
| TRFK301/4 | 18.21±0.10 | 17.21±0.66 | 4.2200 | 0.3260 |
| EPKTN14-3 | 21.32±0.55 | 20.17±0.51 | 2.9200 | 0.8410 |

*Significant mean differences are indicated with **
*TRFK- Tea Research Foundation Kericho*

### 4.4.8 Soil copper (Cu) levels (mg)

Differences in mean control and mean infected copper was assessed for all the six clones. All the six clones showed no significant difference between the infected and the control (Table 4.28).

Table 4.28: Soil copper levels

| | Mean | | | |
|---|---|---|---|---|
| **Clones** | **Infected** | **Control** | **t-test** | **p value** |
| TRFK430/90 | 6.01±0.55 | 6.11±0.57 | 1.7900 | 0.2170 |
| TRFK31/8 | 7.21±0.55 | 6.10±0.01 | 3.8200 | 0.3210 |
| TRFK306/1 | 8.22±1.05 | 7.50±0.10 | 1.1900 | 0.1320 |
| TRFK303/577 | 6.12±0.55 | 7.21±1.00 | 1.3400 | 0.5430 |
| TRFK301/4 | 6.21±0.10 | 7.21±0.66 | 1.2200 | 0.3280 |
| EPKTN14-3 | 8.32±0.55 | 7.17±0.51 | 1.9200 | 0.241 |

*Significant mean differences are indicated with **
*TRFK- Tea Research Foundation Kericho*

### 4.4.9 Soil iron (Fe) levels (mg)

Differences in mean control and mean infected iron was assessed for all the six clones. All the six clones showed no significant difference between the infected and the control (Table 4.29)

Table 4.29: Soil iron levels

| | Mean | | | |
|---|---|---|---|---|
| **Clones** | **Infected** | **Control** | **T-test** | **p value** |
| TRFK430/90 | 70.01±0.55 | 62.11±0.57 | 3.7900 | 0.2470 |
| TRFK31/8 | 68.01±0.55 | 65.1±0.01 | 3.4200 | 0.2210 |
| TRFK306/1 | 66.22±1.05 | 68.0±0.10 | 3.1900 | 0.3320 |
| TRFK303/577 | 65.12±0.55 | 66.21±1.00 | 2.3400 | 0.7430 |
| TRFK301/4 | 73.21±0.10 | 77.21±0.66 | 1.4200 | 0.6280 |
| EPKTN14-3 | 98.32±0.55 | 97.17±0.51 | 1.6200 | 0.2410 |

*Significant mean differences are indicated with **
*TRFK- Tea Research Foundation Kericho*

### 4.4.10 Soil sodium (Na) levels (mg)

Differences in mean control and mean infected N was assessed for all the six clones. All the six clones showed no significant difference between the infected and the control mean soil sodium concentration (Table 4.30).

Table 4.30: Soil sodium levels

| | Mean | | | |
|---|---|---|---|---|
| **Clones** | **Infected** | **Control** | **T-test** | **p value** |
| TRFK430/90 | 348.01±0.55 | 349.11±0.57 | 2.3400 | 0.2410 |
| TRFK31/8 | 358.01±0.55 | 353.1±0.01 | 1.6200 | 0.2470 |
| TRFK306/1 | 350.22±1.05 | 351.0±0.10 | 1.4200 | 0.3320 |
| TRFK303/577 | 340.12±0.55 | 339.21±1.00 | 3.7900 | 0.2210 |
| TRFK301/4 | 372.21±0.10 | 372.21±0.66 | 3.1900 | 0.6280 |
| EPKTN14-3 | 331.32±0.55 | 330.17±0.51 | 3.4200 | 0.7430 |

*Significant mean differences are indicated with **
*TRFK- Tea Research Foundation Kericho*

# CHAPTER FIVE

# DISCUSSION

## 5.1 Effects of *Cuscuta campestris* on tea growth parameters

There were six clones tested for mean difference between infected and control for the shrub-trunk diameter. Among them, only TRFK306/1 showed a significance difference between infected and control. A study by Saric-Skranomovic (2018), on the diameter of central cylinder of alfafa infested with *Cuscuta* spp. showed the lowest % insignificant reduction and during early stages of infection, the host responded with specific gene expression for parasite cell elongation and changes in its cell wall, causing possible increase in shrub diameter. The effects of *Cuscuta* spp. on cultivated hosts is still mostly uninvestigated (Saric-Skranomovic, 2018)

The findings of this study indicated that the leaf area of the infected clone TRFK306/1 and TRFK301/4 was significantly lower than that of the control while all the other clones were not affected. This might either be due to the inhibition in host leaf expansion or on the other hand reduction in the number of leaves or both (Watling and Press, 2001). The findings are also in consistence with those of Shen *et al*. (2005) where *Cuscuta campestris* had an effect on the leaf area of its host *Mikania micrantha.* This indicates that *Cuscuta campestris* may be aggressive on some selected tea clones upon infection e.g. in leaves of clones 306/1 and 301/4 compared to leaves of other clones. Shen *et al*. (2005) observed that infected *M. micrantha* plants playing a host to *Cuscuta* spp. allocated more resources to leaves. However, such increased allocation of resources by host to leaves does not increase the leaf area. This might be caused by parasite capability to inhibit leaf expansion and reduce

remobilization of resources from the infected leaves or both at the beginning of the infection which similarly can be attributed to changes in leaf area of the infected tea clones.

*Cuscuta campestris* significantly did not reduce the number of leaves in all tea clones host after parasitisation. This indicates that the clones have a way of defending themselves against intense infestation by parasitic *C. campestris*. In line with a study by Omar *et al*. (2019), the number of leaves/plants showed a slight non-significant small negative correlation with the infestation level of dodder in Fahl ecotype of the Egyptian clover. In comparison to other studies by Shen *et al*. (2005) on influence of the obligate parasite *Cuscuta campestris* on growth and biomass allocation of its host *Mikania micrantha, Cuscuta campestris* significantly reduced the number of leaves. Shen *et al.* (2011) concluded that *C. campestris* parasitism suppressed host photosynthesis, captured host resources and consequently slowed host growth. This could cause reduced stomatal conductance, transpiration rates, chlorophyll content and rubisco concentration of an invasive host species especially in *M. micrantha,* leading to a decrease in the photosynthesis and growth of the infected host plants. *Cuscuta campestris* was found to affect the masses of above ground and below ground tissues of all the 6 tea clones except for above ground tissue mass of clone TRFK306/1.

The above and below ground mass tissues were higher in the infected than the controls. Contact of *C. campestris* with the host stimulates the development of haustoria that forms connections between the vascular bundles and the host (Kaiser, 2015) increasing stem and root biomass. The host also would develop wound tissue on the area of infection as defense mechanism preventing the establishment of cytoplasmic connection between the host and the parasite contributing to an increase in biomass of roots and stem. The biomass of *C.*

*campestris* was higher in the clones representing wet regime compared to clones representing dry regime.

### 5.2 Effects of *Cuscuta campestris* on tea harvest biomass (g)

All the six clones showed a significant difference in harvest biomass. Moreover, the findings of this research indicated that dry mass of *C. campestris* plus host was less than that of uninfected clones. This is attribute to the powerful metabolic sink effect of *Cuscuta* spp. on its host where the damage to infected hosts can be severe, to the extent of total crop loss. In comparison to other plants such as lucerne, harvest biomass can be reduced significantly with infestation with *C. campestris*. This is consistent with Shen *et al.* (2005) observation that the relationship between *Striga hermonthica* and *C. campestris* led to depression of biomass accumulation in infected plants.

The results are in line with those of Westwood *et al*. (2009) that *Cuscuta* spp. parasitic plants are able to withdraw water, carbohydrate and other soluble materials from a susceptible host. *Cuscuta* spp. operating as a "super-sink" overcomes the host's sinks system resulting in heavy loss in terms of yield.

In line with Koskela *et al*. (2001), *Cuscuta* spp. parasitize many different plants, induce negative impacts on the growth and yield of infested hosts and have significant effects on the structure and functioning of plant communities that are infected by these holoparasites. In other studies, Mishra *et al.* (2007) compared different crops' susceptibility to *Cuscuta* spp. invasion reporting yield losses of 27% in black gram, 48% in soya bean and 82% in green gram in India. In a similar comparative study, legumes were ranked as highly susceptible suffering greater than 50% loss according to Farah and Al-Abdulsalam (2004).

### 5.3 Effects of *Cuscuta campestris* on tea quality

*Cuscuta campestris* did not have any significance effect on % GA, % GC, % EGC, % +C, % EGCg, % EC, % GCg, % Ecg, % Cg, %Total Catechins, % Total gallate Catechins, Gallate ratio (%), % caffeine catechins and catechins caff./cat. ratio (%) of all tea clones under study. All parameters of tea quality were less sensitive indicators of stress caused by *C. campestris*. *Cuscuta* spp. is a strong sink to redirect the flow of host resources to parasites and alters host plants photosynthesis and transpiration. *Cuscuta* spp. infection on host plants chemical components is still unknown (Saric-Krsmanovic *et al.,* 2018). According to Saric-Krismanovic *et al*. (2018), *Cuscuta* affected some chemical components of its host while leaving others unaffected. For example, in peppermint, the contents of the predominant oxygenated monoterpenes were high and very similar in infested and non-infested plants.

In peppermint, *Cuscuta* spp. infestation minor oil constituents, leading to a reduction of about 67% contents of pulegone. According to Saric-Krsmanovic (2020), *Cuscuta spp*. reduce lipid composition in host plants (*Lantana camara*, *Helianthus annuus*, *Medicago sativa* and *Pisum sativum*) upon infestation. In their research, Saric-Krismanovic *et al*. (2018), menthol and menthone remained the main components of peppermint essential oil, irrespective of the *Cuscuta* spp. attack. In addition, Mishra and Sanwal (1992) cited in Saric-Krsmanovic (2020) observed changes in Brassica *juncea* seed oil lipid composition due to *C. reflexa* infection.

### 5.4 Effects of *Cuscuta campestris* on soil chemical characteristics

The findings established no significance differences in soil chemical components levels for pH, potassium, Calcium, Magnesium, Phosphorus, Manganese, Zinc, Copper, iron, sodium

for normal and control. According to a study by Press *et al*. (1999) the extent of parasites competing with the host for carbon and other nutrients depends on their relative sink strength and the degree of autotrophy of the parasite. Stressed plants may be surprisingly good hosts because they possess more concentrated stockpiles of metabolites that are not allocated to growth, and because they accumulate additional carbon and nitrogen containing compounds (Saric-Krsmanovic, 2018). According to a study by Hibberd andJeschke (2001) nitrogen intake by parasite depends primarily on its availability and translocation through the conducting tissue of its host. In the early stages of infestation by *Cuscuta* the host plant reacts with a specific gene expression for calcium release (Werner *et al*., 2001; Albert *et al*., 2004).

# CHAPTER SIX

# CONCLUSIONS AND RECOMMENDATIONS

## 6.1 Conclusions

The present results indicate that the leaf area of the infected clone TRFK306/1 was significantly lower compared to that of control while all the other clones were not affected, which could be due to the inhibition in leaf expansion of surface area or to the reduction in the number of leaves or both. The number of leaves was not reduced by *C. campestris* infection proofing a presence of defensive mechanism against intense infestation by parasitic *C. campestris*.

The present results also indicated that dry mass of *C. campestris* with host was less than that of uninfected clones owing to the powerful metabolic sink effect of *C. campestris* on its host where the damage to infected hosts can be severe. Differences in root biomass in mean control and mean infected clones were assessed for all the six clones. All the six clones showed a significant difference. The mean leaf biomass of TRFK430/90 clone, was affected by *C. campestris* infestation as compared to other clones.

Harvest biomass of all tea clones was affected by *C. campestris* infestation with infected clones having significantly higher biomass. *Cuscuta campestris* did not have any significance effect on % GA, % GC, % EGC, % +C, % EGCg, % EC, % GCg, % ECg, % Cg, %Total Catechins, % Total gallate Catechins, Gallate ratio (%), % Caffeine Catechins and catechins caff./Cat. ratio (%) of the all tea clones under study which could be attributed to in build defensive mechanism reducing withdrawal of tea chemicals by *Cuscuta* haustorium.

The findings established no significance difference in soil chemical components levels for potntial of hydrogen (pH), potassium (K), calcium (Ca), magnesium (Mg), Phosphorus (P), manganese (Mn), zinc (Zn), copper (Cu), iron (Fe) and sodium (Na) for normal and control. The extent of parasites competing with hosts for carbon and other nutrients depends on their natural sink strength and the degree of autotrophy of the parasite. In conclusion, *C. campestris* does not affect nutrients uptake by the host plant.

**6.2 Recommendations**

i. Research should be conducted to assess other contributing factors leading to variable attack of tea by *Cuscuta campestris*.

ii. Cross breeding of clones especially TRFK306/1 with other more resistant clones would probably make TRFK306/1 more resistance to attack.

iii. Research on identification of proteins or genes with key functions in mediating susceptibility of host or resistance to *Cuscuta campestris* infestation on tea clones is recommended.

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# APPENDICES

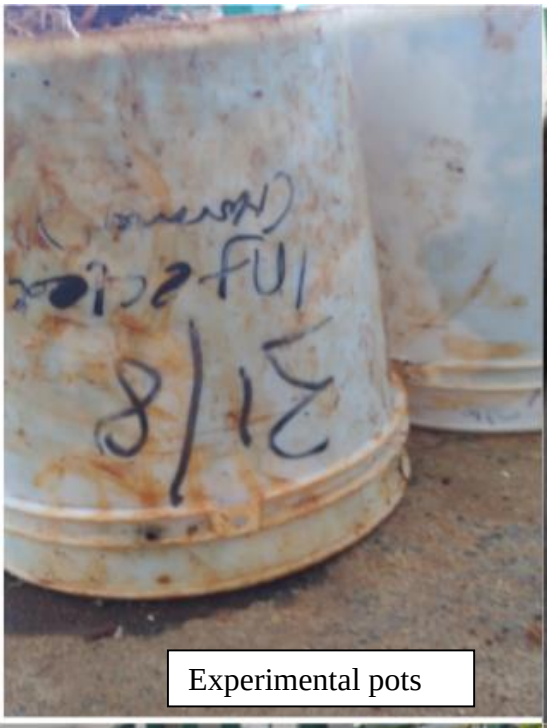
## Appendix I: Data Sheet for tea clones chemical quality

| SAMPLE ID. | | % GA | % GC | % EGC | % +C | % EGCg | % EC | % GCg | % ECg | % Cg | %Total(8) Catechins | % Total gallate | Gallate Ration(%) | % Caffeine | Caff./Cat. Ration(%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SAMPLE NO. 1 | | 0.00 | 0.48 | 4.20 | 0.15 | 10.36 | 0.88 | 0.18 | 1.74 | 0.06 | **18.05** | 12.34 | **68.37** | 3.92 | **21.71** |
| | | 0.00 | 0.48 | 4.20 | 0.15 | 10.32 | 0.88 | 0.19 | 1.74 | 0.06 | **18.02** | 12.31 | **68.32** | 3.91 | **21.70** |
| | Av. | **0.00** | **0.48** | **4.20** | **0.15** | **10.34** | **0.88** | **0.19** | **1.74** | **0.06** | **18.04** | **12.33** | **68.34** | **3.92** | **21.70** |
| SAMPLE NO. 2 | | 0.06 | 0.59 | 4.61 | 0.15 | 9.95 | 0.95 | 0.40 | 1.62 | 0.07 | **18.34** | 12.04 | **65.64** | 3.40 | **18.54** |
| | | 0.06 | 0.59 | 4.61 | 0.15 | 9.95 | 0.95 | 0.40 | 1.62 | 0.07 | **18.34** | 12.04 | **65.64** | 3.40 | **18.54** |
| | Av. | **0.06** | **0.59** | **4.61** | **0.15** | **9.95** | **0.95** | **0.40** | **1.62** | **0.07** | **18.34** | **12.04** | **65.6** | **3.40** | **18.54** |
| SAMPLE NO. 3 | | 0.00 | 0.30 | 3.36 | 0.28 | 6.54 | 1.95 | 0.14 | 2.58 | 0.00 | **15.15** | 9.26 | **61.13** | 2.64 | **17.43** |
| | | 0.00 | 0.30 | 3.35 | 0.28 | 6.54 | 1.94 | 0.14 | 2.58 | 0.00 | **15.13** | 9.26 | **61.20** | 2.64 | **17.45** |
| | Av. | **0.00** | **0.30** | **3.36** | **0.28** | **6.54** | **1.95** | **0.14** | **2.58** | **0.00** | **15.14** | **9.26** | **61.17** | **2.64** | **17.44** |
| SAMPLE NO. 4 | | 0.00 | 0.44 | 5.09 | 0.13 | 5.37 | 1.10 | 0.10 | 1.08 | 0.00 | **13.31** | 6.55 | **49.20** | 1.82 | **13.68** |
| | | 0.00 | 0.44 | 5.09 | 0.13 | 5.37 | 1.11 | 0.10 | 1.08 | 0.00 | **13.32** | 6.55 | **49.17** | 1.82 | **13.67** |
| | Av. | **0.00** | **0.44** | **5.09** | **0.13** | **5.37** | **1.11** | **0.10** | **1.08** | **0.00** | **13.31** | **6.55** | **49.19** | **1.82** | **13.67** |
| SAMPLE NO. 5 | | 0.00 | 0.36 | 4.65 | 0.20 | 9.39 | 1.70 | 0.25 | 2.66 | 0.05 | **19.26** | 12.35 | **64.12** | 3.15 | **16.36** |
| | | 0.00 | 0.36 | 4.63 | 0.20 | 9.37 | 1.69 | 0.25 | 2.65 | 0.05 | **19.20** | 12.32 | **64.17** | 3.13 | **16.30** |
| | Av. | **0.00** | **0.36** | **4.64** | **0.20** | **9.38** | **1.70** | **0.25** | **2.66** | **0.05** | **19.23** | **12.33** | **64.14** | **3.14** | **16.33** |
| SAMPLE NO. 6 | | 0.00 | 0.29 | 5.04 | 0.17 | 8.55 | 1.30 | 0.35 | 1.76 | 0.06 | **17.52** | 10.72 | **61.19** | 2.94 | **16.78** |
| | | 0.00 | 0.29 | 5.03 | 0.17 | 8.54 | 1.30 | 0.35 | 1.76 | 0.06 | **17.50** | 10.71 | **61.20** | 2.93 | **16.74** |
| | Av. | **0.00** | **0.29** | **5.04** | **0.17** | **8.55** | **1.30** | **0.35** | **1.76** | **0.06** | **17.51** | **10.72** | **61.19** | **2.94** | **16.76** |
| SAMPLE NO. 7 | | 0.23 | 0.00 | 0.19 | 0.00 | 0.98 | 0.21 | 0.14 | 0.70 | 0.00 | **2.22** | 1.82 | **81.98** | 2.99 | **134.68** |
| | | 0.23 | 0.00 | 0.19 | 0.00 | 0.98 | 0.21 | 0.14 | 0.65 | 0.00 | **2.17** | 1.77 | **81.57** | 2.99 | **137.79** |
| | Av. | **0.23** | **0.00** | **0.19** | **0.00** | **0.98** | **0.21** | **0.14** | **0.68** | **0.00** | **2.20** | **1.80** | **81.77** | **2.99** | **136.24** |
| SAMPLE NO. 8 | | 0.23 | 0.00 | 0.19 | 0.00 | 0.86 | 0.20 | 0.14 | 0.58 | 0.00 | **1.97** | 1.58 | **80.37** | 3.02 | **153.61** |
| | | 0.23 | 0.00 | 0.19 | 0.00 | 0.86 | 0.20 | 0.14 | 0.62 | 0.00 | **2.01** | 1.62 | **80.60** | 3.00 | **149.25** |
| | Av. | **0.23** | **0.00** | **0.19** | **0.00** | **0.86** | **0.20** | **0.14** | **0.60** | **0.00** | **1.99** | **1.60** | **80.48** | **3.01** | **151.43** |
| | | 0.19 | 0.08 | 0.28 | 0.08 | 0.84 | 0.27 | 0.18 | 0.60 | 0.00 | **2.32** | 1.62 | **69.71** | 2.47 | **106.28** |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SAMPLE NO. 9 | | 0.19 | 0.08 | 0.28 | 0.08 | 0.84 | 0.28 | 0.18 | 0.60 | 0.00 | **2.33** | 1.62 | **69.44** | 2.47 | **105.87** |
| | Av. | **0.19** | **0.08** | **0.28** | **0.08** | **0.84** | **0.28** | **0.18** | **0.60** | **0.00** | **2.33** | **1.62** | **69.57** | **2.47** | **106.08** |
| SAMPLE NO. 10 | | 0.22 | 0.12 | 0.32 | 0.11 | 0.97 | 0.37 | 0.17 | 0.77 | 0.05 | **2.88** | 1.96 | **68.03** | 2.52 | **87.56** |
| | | 0.22 | 0.12 | 0.32 | 0.11 | 0.97 | 0.37 | 0.17 | 0.77 | 0.05 | **2.88** | 1.96 | **68.03** | 2.52 | **87.56** |
| | Av. | **0.22** | **0.12** | **0.32** | **0.11** | **0.97** | **0.37** | **0.17** | **0.77** | **0.05** | **2.88** | **1.96** | **68.03** | **2.52** | **87.56** |
| SAMPLE NO. 11 | | 0.00 | 0.35 | 5.57 | 0.17 | 9.28 | 1.35 | 0.37 | 1.86 | 0.11 | **19.06** | 11.62 | **60.97** | 2.99 | **15.69** |
| | | 0.00 | 0.36 | 5.57 | 0.17 | 9.28 | 1.35 | 0.36 | 1.86 | 0.09 | **19.04** | 11.59 | **60.88** | 2.99 | **15.70** |
| | Av. | **0.00** | **0.36** | **5.57** | **0.17** | **9.28** | **1.35** | **0.37** | **1.86** | **0.10** | **19.05** | **11.61** | **60.92** | **2.99** | **15.69** |
| SAMPLE NO. 12 | | 0.00 | 0.55 | 5.50 | 0.19 | 8.36 | 1.16 | 0.14 | 1.59 | 0.14 | **17.63** | 10.23 | **58.03** | 2.66 | **15.09** |
| | | 0.00 | 0.55 | 5.50 | 0.19 | 8.36 | 1.17 | 0.14 | 1.58 | 0.14 | **17.63** | 10.22 | **57.97** | 2.65 | **15.03** |
| | Av. | **0.00** | **0.55** | **5.50** | **0.19** | **8.36** | **1.17** | **0.14** | **1.59** | **0.14** | **17.63** | **10.23** | **58.00** | **2.66** | **15.06** |
| SAMPLE NO. 13 | | 0.07 | 0.04 | 1.00 | 0.07 | 3.48 | 0.83 | 0.24 | 2.40 | 0.28 | **8.34** | 6.40 | **76.76** | 1.93 | **23.15** |
| | | 0.07 | 0.04 | 1.00 | 0.07 | 3.47 | 0.82 | 0.23 | 2.39 | 0.29 | **8.31** | 6.38 | **76.80** | 1.93 | **23.23** |
| | Av. | **0.07** | **0.04** | **1.00** | **0.07** | **3.48** | **0.83** | **0.24** | **2.40** | **0.29** | **8.32** | **6.39** | **76.78** | **1.93** | **23.19** |
| SAMPLE NO. 14 | | 0.00 | 0.05 | 1.82 | 0.04 | 2.67 | 0.85 | 0.04 | 0.99 | 0.12 | **6.58** | 3.82 | **58.08** | 1.58 | **24.00** |
| | | 0.00 | 0.05 | 1.82 | 0.05 | 2.67 | 0.85 | 0.04 | 0.99 | 0.11 | **6.57** | 3.81 | **57.97** | 1.58 | **24.04** |
| | Av. | **0.00** | **0.05** | **1.82** | **0.04** | **2.67** | **0.85** | **0.04** | **0.99** | **0.12** | **6.58** | **3.82** | **58.03** | **1.58** | **24.02** |
| SAMPLE NO. 15 | | 0.08 | 0.25 | 1.81 | 0.35 | 5.10 | 3.00 | 0.29 | 4.93 | 0.05 | **15.78** | 10.37 | **65.72** | 2.65 | **16.79** |
| | | 0.08 | 0.25 | 1.81 | 0.35 | 5.09 | 3.00 | 0.28 | 4.92 | 0.06 | **15.76** | 10.35 | **65.66** | 2.65 | **16.82** |
| | Av. | **0.08** | **0.25** | **1.81** | **0.35** | **5.10** | **3.00** | **0.29** | **4.93** | **0.05** | **15.77** | **10.36** | **65.69** | **2.65** | **16.81** |
| SAMPLE NO. 16 | | 0.00 | 0.12 | 1.57 | 0.23 | 2.57 | 3.62 | 0.09 | 2.95 | 0.00 | **11.15** | 5.61 | 50.31 | 1.67 | **14.98** |
| | | 0.00 | 0.12 | 1.56 | 0.23 | 2.56 | 3.58 | 0.09 | 2.94 | 0.00 | **11.08** | 5.59 | 50.44 | 1.66 | **14.98** |
| | Av. | **0.00** | **0.12** | **1.57** | **0.23** | **2.57** | **3.60** | **0.09** | **2.95** | **0.00** | **11.11** | **5.60** | **50.38** | **1.67** | **14.98** |

Analysis of catechin content carried out using Agilent HPLC 1260: catechin @ 230nm and caffeine @ 275nm.

**Appendix II: List of plates showing research on the field**



Experimental pots

Data collection

Data collection

Above ground tissue

Infestation

Cuscuta plant

Above ground tissue

Below ground tissue

Clones ready for infestation

Cuscuta infestation on tea

## Appendix III: Similarity Report

Turnitin Originality Report

THE EFFECTSOF CuscutacampestrisYunckINVASION ON TEA GROWTH PARAMETERS, QUALITY AND SOIL NUTRIENT UPTAKEINNANDI,KENYA by Monica Jemaiyo

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