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Influence of Some Plant Extracts on Physiological Traits of French Beans (*Phaseolus vulgaris* L.) Infected with Rust (*Uromyces appendiculatus*)

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ABSTRACT

Plant growth analysis is a standard approach to study plant growth and productivity. The following plants Boscia angustifolia, Zanthoxylum chalybeum and Melea volkensii were selected based on their antifungal activities on rust from previous experiments. The extracts were used as single treatments and combined with each other namely; Z. chalybeum- M. volkensii, B. angustifolia - Z. chalybeum and B. angustifolia - M. volkensii making a total of eight treatments including a negative and positive controls. Soil analysis of study site was conducted. Plant growth parameters that include plant shoot height and dry shoot weight were used to assess the effects of various treatments on bean plant performance. Ten plants sampled in each of the four plots in a treatment were uprooted and sampled leaves plucked and area measured. In this study the plots where the French beans were grown had nitrogen and phosphorous deficiency. Phosphorus was in low levels (5.4mgkg-1) which was appropriate for French beans hence it was necessary to supply fertilizer of right amounts to supplement the deficiency. Commercial fungicide (Kocide DF) treatment did not affect the leaf area but Z. chalybeum treatment had the lowest mean leaf area throughout the growth period. This may suggest that the commercial fungicide can be tolerated by the plants. Z. chalybeum treatment may have had some secondary metabolites that could have been harmful to the bean plants. B. angustifolia –Z. chalybeum, M. volkensii and Kocide DF treatments had highest mean dry shoot weights throughout the growth period meaning they were friendly to the physiological processes. Untreated control and Z. chalybeum had lower mean dry shoot weights; this could be because of the higher disease severity and incidence. Commercial fungicide, untreated control and Z. chalybeum treatments had lower mean shoot heights compared to other treatments. This indicated that apart from rust disease causing low shoot height in untreated control, Kocide DF and Z. chalybeum treatments could have initiated production of compounds that deterred increase in shoot height.

Key words: *antifungal; dry shoot weight; shoot height; leaf area*

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INTRODUCTION

French beans (*Phaseolus vulgaris* L.) are important in the farming systems of East and Central Africa. They are also called Haricot beans, string beans, snap beans or fillet beans and belong to the family of plants called Leguminosae (Fabaceae). The crop has great potential for addressing food security, income generation and poverty alleviation . In 2009, Kenya produced 29,923 metric tonnes of vegetables valued at KES 4.2 billion that were marketed to various destinations as fresh produce and processed products. French beans accounted for a significant proportion of total horticultural exports.

Bean rust, caused by the fungus Uromyces appendiculatus, is a common and serious disease of French beans worldwide but is most prevalent in tropical and sub-tropical areas. It causes 25 - 100% yield loss depending on the stage of infection and the prevailing weather conditions. Kenyan French beans are largely exported to the European markets where consumers demand aesthetic quality products that are disease free. This has generally encouraged excessive use of chemical pesticides in French bean production in Kenya. Chlorothalonil and copper based fungicides have been effective in the control of bean rust but indiscriminate use of these chemicals has often resulted in adverse environmental effects, development of pest resistance and negative effects on human health. Concerns over the adverse effects of chemical fungicides on the health of consumers have lead to revision of food safety standards in regard to pesticide residue in fresh produce.

The revision of international food safety standards has introduced a new order in the use of pesticides in production of fresh vegetables destined for markets in developed countries. Alternative non-chemical disease management strategies which are based on naturally occurring compounds need to be developed to ensure safe trading. A possible alternative is the use of antifungal plant extracts. The plant world comprises a rich storehouse of biochemicals that can be used as biological pesticides which are environmentally safe. Extracts from plant such as Melia azedarach, *Euclyptus* citriodora, Azadirachta indica, Allium sativum, Lippea javanicum, Urtica massaica, Satureia biflora, Warburgia ugandensis, Zingiber officinales *and* Alstonia scholaris *have* showed antimicrobial activity against a wide range of plant pathogens. *B. angustifolia, M. volkensii, and Z. chalybeum* have been shown to have antifungal activities against bean rust (*Uromyces appendiculatus*) from previous experiments[7].

MATERIALS AND METHODS

Study site

Experiments were carried out in the field at Jomo Kenyatta University of Agriculture and Technology (JKUAT) in Thika District. The university is located at latitude 1°05° S and longitude 37°00° E. It lies at an altitude of 1525 meters above sea level and it receives an annual rainfall of 850mm. Temperatures range from 13°C and 26°C.

Collection and processing of plant materials

The samples of three desired plants from previous studies[6,10] that showed antifungal activity were collected from different parts of the country (Samburu, Mombasa, Mwingi, Kakamega forest and Nakuru) in clean sacks (Table 1). The plants were stored at Jomo Kenyatta University of Agriculture and Technology (Taxonomy unit, Department of Botany). Voucher specimens were deposited in the Herbarium. The samples were labeled and deposited in the GK Botany laboratory at a room temperature for three weeks. The plant leaves and roots were dried separately at room temperature for a period of 3 weeks and then ground separately to powder using a grinding mill at 8000rpm (Type 8 lab mill). The powder was stored in plastic bags at room temperature until the time required.

Table 1. Selected plants for the study and parts of the plants used

Family	Scientific Name	Common/local name	Parts used
Capparidaceae	*Boscia angustifolia*	Mulule (Kamba)	Leaves, Stem
Rutaceae	*Zanthoxylum chalybeum*	Mjafari (Swahili)	Leaves, Stem
Rutaceae	*Melea volkensii*	Mukau (Kamba)	Leaves, Stem

Field experiment

Study site soil analysis

Soil analysis of study site was conducted. Sample comprised of approximately 1 kg of soil taken from a depth of 6 inches (15 cm). At least 25 cores were taken at random from each area to be sampled and together formed a single representative sample. All cores were mixed thoroughly and a sub-sample taken to the laboratory. Soil analysis for nitrogen was done using Kjeldahl digests by titration, steam distillation and colorimetric methodologies, while phosphorus concentration was determined using sodium acetate, Bray, sodium bicarbonate (Olsen), ammonium bicarbonate-DTPA (Diethylene triamine Pentaacetic Acid), Morgan extracting solutions12. Potassium and sodium were determined by atomic absorption spectrophotometry. Zinc and copper were determined using DTPA and ammonium bicarbonate-DTPA extracting solutions. Organic matter was determined following combustion[12].

Field layout and planting

Seeds were obtained from Regina Seed Company and planted at a spacing of 30cm between rows and 10cm between plants within the rows8. French bean seeds commercially available coated with thiram were used to control root rots. French bean variety Amy seeds were planted in 4×3m plots each separated by a 1m path between the treatments and the replications. Amy is high yielding compared to other varieties therefore it is grown by most farmers. Di-ammonium phosphate (DAP) was used at planting at a rate of 200kg/ha mixed well before seed placement. Calcium ammonium nitrate (CAN) was applied at a rate of 100Kg/ha at trifoliate leaf stage.

Treatment application

The best three extracts in the in vitro and greenhouse experiments from previous studies namely B. angustifolia, M. volkensii, and Z. chalybeum were used in the field experiment using bean rust natural inoculums (spores dispersed by wind naturally found in the field where previously beans were planted). The percentage uredospore germination and disease severity were used to determine the best extracts in bean rust control. The extracts were used as single treatments and combined with each other namely; *Z. chalybeum*- *M. volkensii*, *B. angustifolia* - *Z. chalybeum* and *B. angustifolia* - *M. volkensii* making a total of eight treatments including a negative and positive controls.

The treatments consisted of six plant extracts, copper hydroxide 61.4% (Kocide DF: metallic copper equivalent 40% formulated as a dry flowable) as a positive control and a negative water control. Combinations of powders in a 1:1 ratio were soaked in water overnight and strained. Two kilogram of each plant sample was soaked in ten liters of water and left overnight to allow extraction of the crude active compounds. The supernatant of each plant extract was filtered in several layers of muslin cloth and volumes adjusted to 20 L[15]. A bar soap ground to powder and dried was used as a sticker at a rate of 1 g per litre of water extracts. A spray regime of once a week using a knap sack was employed from the fifteen days after planting until flowering. The extracts used were used as protectants. The fungicide was applied at a rate of 2.5kg ha^{-1} according to the manufacturers' recommendations. Eight treatments were replicated four times in a randomized block design (RBD) making a total of thirty two plots. There were a total of seven hundred and sixty plants per replicate. Overhead irrigation twice a week and weeding were done as necessary.

Biomass measurements

Biomass measurements were done only in the field experiment. Ten representative plants from each of the eight treatments were randomly harvested after three weeks of planting French beans to estimate the initial biomass. Plant growth parameters that include plant shoot height and dry shoot weight were used to assess the effects of various treatments on bean plant performance.

Shoot height was taken from the first node to the leaf apex where ten plants were randomly selected in each plot making a total of forty plants per treatment, marked and shoot heights taken once every month from the start of foliar (six weeks after planting) sprays until flowering. The plants were then harvested and dried separately in an oven at 80°C for 72 h. The dry shoot weights were recorded on ten sampled plants from each treatment.

Leaf area measurement

Ten plants sampled in each of the four plots in a treatment were uprooted and sampled leaves plucked and area measured. This was done at the 1st, 2nd and 3rd months of plant growth. Leaf area for the ten sampled plants from each plot was used to estimate the area. A total of one hundred and twenty plants per treatment were sampled at three intervals of three months during the season. A total of two hundred and forty plants were sampled for the experiment. These methods involved the use of leaf area meter[9]. The mean area of the ten sampled leaves was multiplied by the total number of leaves present to provide an estimate of the total leaf area for each plant. The aim was to establish whether treatments had impact on leaf expansion.

RESULTS

Evaluation of field soil for its nutritional status

The baseline values for the soil characteristics at the start of the field experiment were recorded. There were 13.6 mgkg-1 of copper (Cu), 199.1mgkg-1 of Zinc (Zn), 120.5 mgkg-1 of sodium (Na), 818.513.6 mgkg-1 of potassium (K), 5.4 mgkg-1 of Phosphorus (P) and 15.0 mgkg-1 of Nickel (Ni) ions present in the soil. The soil had a higher organic matter content recording 30300 mgkg-1; however there was no nitrogen (N) found.

Effect of leaf area

In the 1st month of growth, there were significant differences among the treatments (P=0.0199). Mean leaf area in the 1st month were; 10.81±1.4 for *B. angustifolia* and 7.47±0.7 for Kocide DF. *Z. chalybeum*, *B. angustifolia - Z. chalybeum*, untreated control had a mean leaf area of 10.46±1.2, 10.03±0.9 and 10.01±0.9 respectively. *B. angustifolia -M. volkensii* (8.35±1.0) had the second lowest mean leaf area (8.35±1.0) (Table 2).

Table 2. Mean leaf area for sampled plants within each treatment in 1st, 2nd and 3rd months of plant growth

	Leaf area (cm^2) per plant			Mean Total leaf
Treatments	**1st month**	**2nd month**	**3rd Month**	**area per plant**
B. angustifolia	*10.81±0.003[a]	41.66±0.003[g]	3000.3±0.200[d]	3052.97±0.049[d]
Z. chalybeum	10.43±0.031[b]	41.65±0.050[g]	2711.6±0.100[h]	2763.70±0.100[h]
B. angustifolia -Z. chalybeum	10.04±0.010[c]	59.52±0.011[a]	2714.9±0.250[g]	2784.19±0.049[g]
Untreated control	10.01±0.001[c]	44.33±0.020[e]	3115.5±0.150[c]	3169.76±0.000[c]
Z. chalybeum -M. volkensii	9.78±0.004[d]	54.43±0.002[b]	3206.4±0.400[b]	3270.38±0.162[b]
M. volkensii	9.00±0.002[e]	44.03±0.007[f]	2783.5±0.150[f]	2836.59±0.146[f]
B. angustifolia -M. volkensii	8.35±0.004[f]	50.55±0.021[c]	3262.5±0.200[a]	3321.28±0.057[a]
Kocide DF	7.45±0.018[g]	46.07±0.006[d]	2841.1±0.200[e]	2894.43±0.003[e]
LSD	0.0433	0.0689	0.7269	0.3240
CV%	0.1978	0.0625	0.0106	0.0043

*Numbers represent mean leaf area for each treatment at time intervals.

*Means separated using LSD test by the same letter along the column are not significantly different (P<0.05) from each other.

There were significant differences in the mean leaf area of treatments (P=0.0003) in the 2nd month. *B. angustifolia - Z. chalybeum* treated bean plants had the highest mean leaf area (59.52±0.011) while *Z. chalybeum* sprayed plots had the lowest mean leaf area (41.65±0.050). *Z. chalybeum - M. volkensii* (54.43±0.002), *B. angustifolia - M. volkensii* (50.55±0.021) and Kocide DF (46.07±0.006) treatments had average mean leaf area. Plots sprayed with untreated control, *M. volkensii*, *B. angustifolia* and *Z. chalybeum* recorded lower leaf areas (Table 2).

The 3rd month of growth experienced no significant differences within the treatments (P=0.2327). Mean leaf area of treatments ranged from *B. angustifolia - M. volkensii* sprayed plots with the highest (3262.5±0.200) to *Z. chalybeum* treated plots having the lowest mean leaf area (2711.6±0.100).

B. angustifolia, untreated control, *X chalybeum - M. volkensii* and Kocide DF sprayed plots had a mean leaf area of 3000.3±0.200, 3115.5±0.150, 3206.4±0.400 and 2841.1±0.200 respectively (Table 2). *B. angustifolia -Z. chalybeum* and *M.*

volkensii had lower mean leaf area compared to most treatments in the 3rd month indicating 2714.9±0.250 and 2783.5±0.150 respectively. There were no significant differences (P=0.2365) in mean total leaf area of all treatments studied. However, mean total leaf area ranged from *B. angustifolia - M. volkensii* treatments having the highest total leaf area (3321.28 cm^2) followed by *Z. chalybeum - M. volkensii* (3270.38 cm^2), Untreated control (3169.76cm^2), *B. angustifolia (*3052.97 cm^2*),* Kocide DF (2894.43 cm^2), *B. angustifolia - Z. chalybeum* (2784.19 cm^2) and *Z. chalybeum* (2763.70 cm^2) respectively (Table 2).

Effects of plant extracts on shoot height

In the 1st Month there were no significant differences between the treatments (P=0.2048). However, *B. angustifolia – Z. chalybeum* treated plants had the highest mean shoot height (15.21±0.96) while *Z. chalybeum* treated plants had the lowest mean shoot height (11.46±1.09). Kocide DF had the second highest mean shoot height (14.82±1.17) while *M. volkensii* treated plots had the third highest mean shoot height (14.16±1.05). Untreated control was second last in terms of mean shoot height measurements (12.06±1.02). These results are shown in Fig 1.

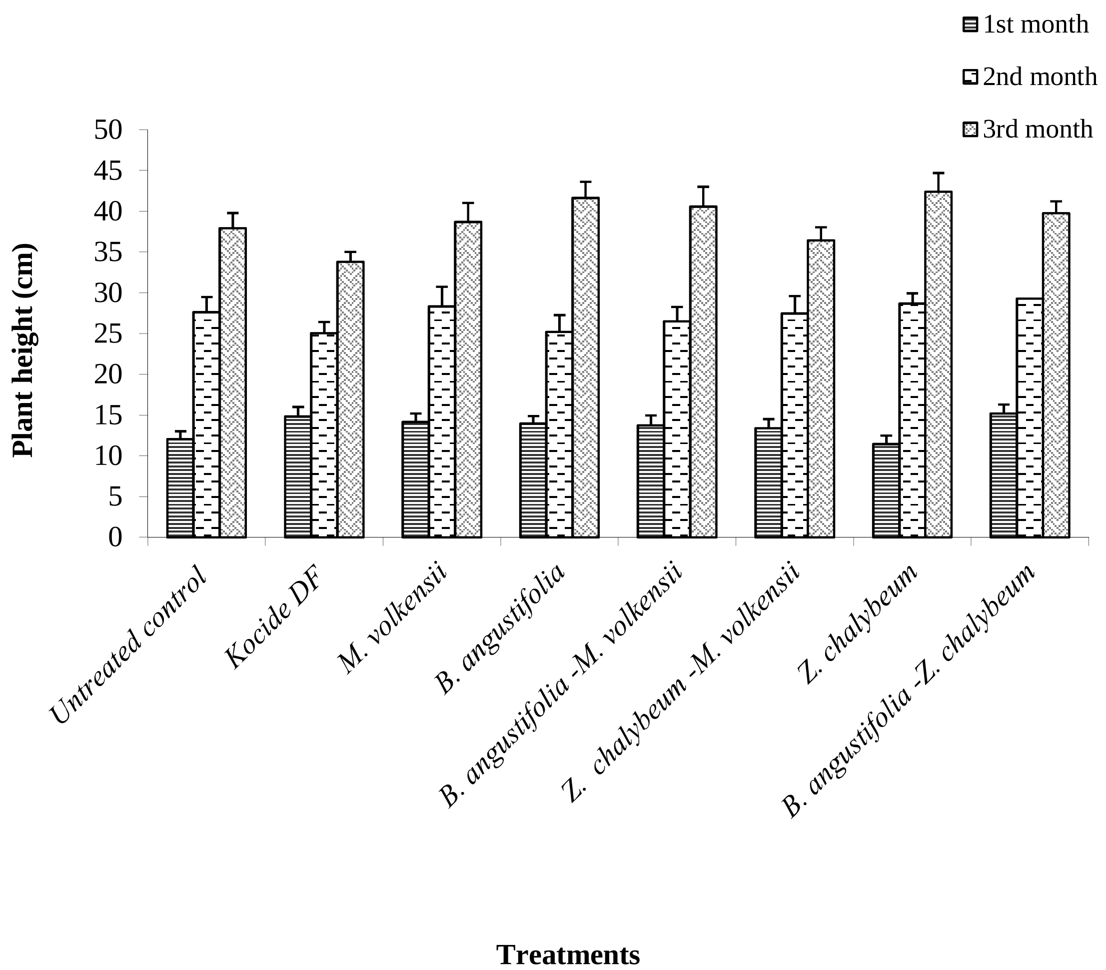

Fig. 1: Mean shoot height of French beans sprayed with selected plant extracts and Kocide DF in the field experiment

In the 2nd month, there were no significant differences in different treatments (P=0.6603) in shoot height. However, plant shoot height differed from *B. angustifolia –Z. chalybeum* treated plants (29.29±1.91) with the highest while Kocide DF treated plants had the lowest mean shoot height (25.04±1.62). *Z. chalybeum* treated plots had the second highest mean shoot height (28.67±1.38) while *B. angustifolia* plants had the second lowest mean shoot height

(25.2±1.27) compared to other treatments (Fig.1). The 3rd month experienced significant differences among the treatments (P<0.05). *Z. chalybeum* treated plants had the highest mean shoot heights (42.41±1.90) compared to other treatments in the 3rd month followed by *B. angustifolia* (41.66±1.21) and *B. angustifolia - M. volkensii* (40.59±2.34) treated plants (Fig. 1). *B. angustifolia – Z. chalybeum* (39.78±1.97), *M. volkensii* (38.69±2.43), *Z. chalybeum - M. volkensii* (36.44±2.31) and untreated control (37.93±1.61) treatments had moderate shoot heights. Kocide DF plots had the lowest mean shoot heights (33.82±1.46) compared to all other treatments. There were no significant differences (P=0.1256) in mean total shoot height of all treatments including the controls (Fig. 1)

Effect of plant extracts on dry shoot weight

There were no significant differences between treatments in the 1st month (P=0.217). However, B. angustifolia had the highest mean dry shoot weight (11.9±1.46) followed by untreated control (9.836±1.30) and the combination B. angustifolia - Z. chalybeum recorded the third highest mean dry shoot weight (8.94±1.98). Z. chalybeum had the lowest mean dry shoot weight (7.856±0.94) among all treatments in the 1st month (Fig. 2). There were significant differences in treatments in the 2nd month (P=0.0279). B. angustifolia - Z. chalybeum (17.952±2.06) and M. volkensii (16.239±2.11) treated plots had the highest mean dry shoot weights compared with other treatments. B. angustifolia (14.8±1.14), kocide DF (14.754±1.58), Z. chalybeum - M. volkensii (14.471±1.51) and B. angustifolia - M. volkensii (13.463±1.57) treatments had better dry shoot weights in the 2nd month of growth than untreated control and Z. chalybeum treated plants. Untreated control (10.336±2.10) and Z. chalybeum (10.115±1.54) treated plots had the lowest mean dry shoot weights compared to other treatments (Fig. 2).

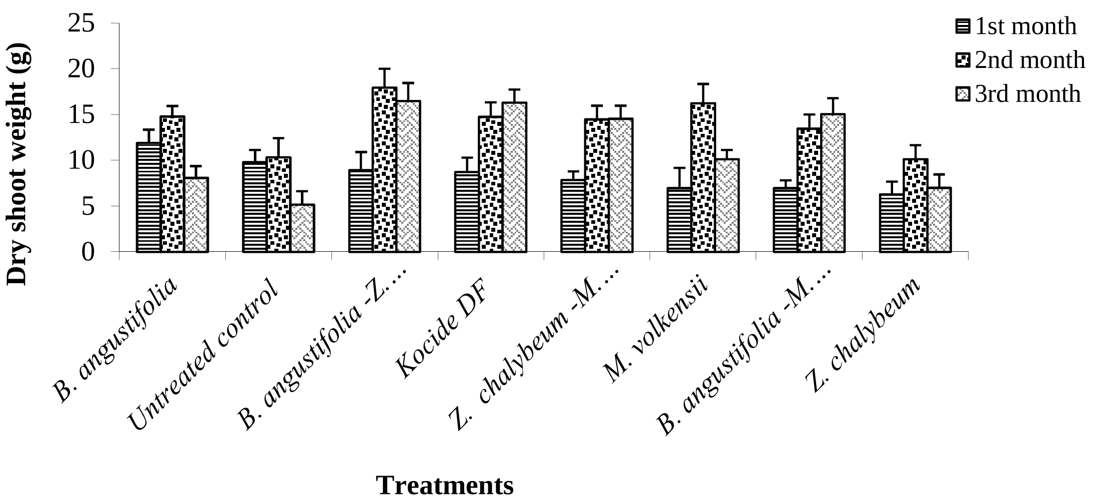

Figure 2. Dry shoot weight of experimental plants for all treatments in a period of three months

In the 3rd month, treatments were significantly different from each other (P<0.0001) in dry shoot weight (Fig. 2). *B. angustifolia* (8.099±1.27), Kocide *DF (*16.31±1.45), *B. angustifolia - M. volkensii (*15.065±1.73) and *Z. chalybeum - M. volkensii (*14.567±1.42) treated plots had the highest mean dry shoot weights of bean plants compared to other treatments (Fig. 7). *M. volkensii* treated plants had higher mean dry shoot weight (10.115±1.04) compared to *B. angustifolia* (8.099±1.27) *and Z. chalybeum* treated plants (6.996±1.45). Untreated control plants had the lowest mean dry shoot weight (5.166±1.46).

There were differences in mean total dry weight of the treatments (P<0.0001). *B. angustifolia* (34.799g), *B. angustifolia –Z. chalybeum* (43.368g), Kocide DF (39.799g), *Z.*

chalybeum - *M. volkensii* (36.894g), *M. volkensii* (33..326g) and *B. angustifolia* - *M. volkensii* (35.488g) treated bean plants had the highest mean total dry weights. Untreated control (25.338g) and *Z. chalybeum* (23.395g) plants had significantly the lowest means of the total dry weight compared to other treatmnts (Fig. 3).

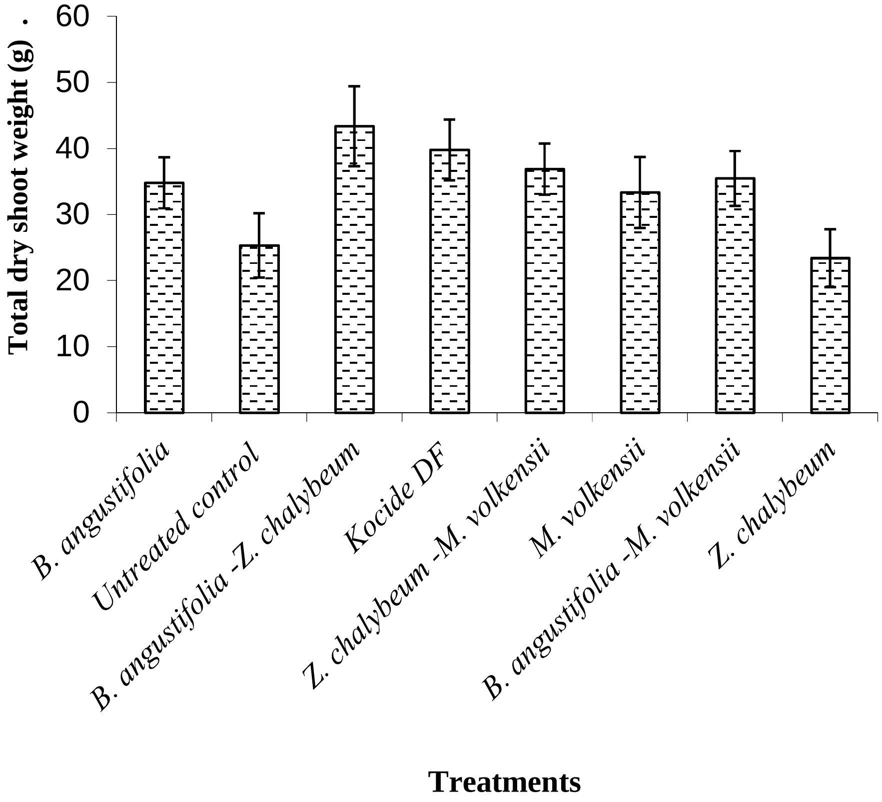

Fig. 3: Mean total dry weight for the sampled bean plants from all treatments

DISCUSSION

Assessing soil characteristics

Trace elements needed in smaller quantities cadmium, copper (13.6 mgkg-1), sodium (120.5mgkg-1) and zinc (199.1 mgkg-1) examined before the field experiment was carried out were in correct amounts. This was done to rule out that any plant symptoms were due to fungal/bacterial/viral but not due to nutrient deficiencies.

Potassium is the third most likely, after nitrogen and phosphorous, to limit plant productivity. For this reason, it is commonly applied to soils as fertilizer and is a component of most mixed fertilizers. For this reason N: P: K fertilizer was applied at the planting and a few weeks after emergence of seedlings. In this study the plots where the French beans were grown had nitrogen and phosphorous deficiency. Phosphorus was in low levels (5.4mgkg^{-1}) which was appropriate for French beans hence it was necessary to supply fertilizer of right amounts to supplement the deficiency. This is because it has been established that high levels of available phosphorus in soil or high application rates of phosphate may induce zinc deficiency in plants grown on soils characterized by low concentrations of available zinc. The interaction of phosphorus and zinc, called phosphorus-induced zinc deficiency, has been observed in many crops, such as bean, wheat, tomato, cotton, flax, soybean, grape and citrus[3,14,17].

Effect of plant extracts on growth parameters

There were no significant differences in leaf area, shoot height and dry shoot weight among the treatments in the 1st month this could be because at seedlings stage the plants had not been naturally inoculated with rust. B.

angustifolia –Z. chalybeum and B. angustifolia – M. volkensii had higher mean leaf area, this indicated better physiological processes. Leaf area is an essential component to estimate plant growth through its incidence on crop physiology mechanisms[2,11].

Commercial fungicide (Kocide DF) treatment did not affect the leaf area but *Z. chalybeum* treatment had the lowest mean leaf area throughout the growth period. This may suggest that the commercial fungicide can be tolerated by the plants. *Z. chalybeum* treatment may have had some secondary metabolites that could have been harmful to the bean plants. Phytotoxin in the form of phenols have been found to have an adverse effect on germination and growth parameters[1,5,13].

Therefore, leaf area measurements for physiological studies is one of the most essential processes, such as one of the physiological determinants of plant growth is the efficiency of the leaves with which the intercepted light energy is used in the production of new dry matter[4,16]. *B. angustifolia –Z. chalybeum*, *M. volkensii* and Kocide DF treatments had highest mean dry shoot weights throughout the growth period meaning they were friendly to the physiological processes. Untreated control and *Z. chalybeum* had lower mean dry shoot weights; this could be because of the higher disease severity and incidence. Siddiqui *et al.*, (1997)[13] reported that benlate (fungicide) caused an increase in fresh and dry weights of *Sesbania sesban* at 0.25g/l concentration. Commercial fungicide, untreated control and *Z. chalybeum* treatments had lower mean shoot heights compared to other treatments. This indicated that apart from rust disease causing low shoot height in untreated control, Kocide DF and *Z. chalybeum* treatments could have initiated production of compounds that deterred increase in shoot height.

The results confirmed Heisy (1990) research that exposure of plants to fungicide creates chemical stress facilitating the production of compounds that are potential inhibitor of germination and seedling growth. *M. volkensii*, *B. angustifolia –Z. chalybeum* and Kocide DF treatments had no effect on the bean pods as compared to untreated control meaning they had less damage.

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