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Effects of crude extracts on some selected physiological parameters of French beans (*Phaseolus vulgaris*) infected with rust (*Uromyces appendiculatus*)

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Rust (*Uromyces appendiculatus*) is a major foliar disease that reduces yield and pod quality in beans. The field trial of French beans was established at Jomo Kenyatta University of Agriculture and Technology (JKUAT). Single plant extracts and combinations of *Boscia angustifolia, Zanthoxylum chalybeum* and *Melea volkensii* were used to evaluate their effect on *U. appendiculatus* in the field. During the growing period, beans were infected with rust from natural inoculum at the field. Physiological responses such as carbon dioxide assimilation, transpiration (E), stomatal conductance (gs), and photosynthetic rate (Pn) of French beans treatments were examined after extracts of three antifungal plants were sprayed. *B. angustifolia - Z. chalybeum* combination and single plant treatment *M. volkensii* had positive effects on enhancing the rate of photosynthesis in bean plants. The high regressions between stomatal conductance and rate of transpiration in the treatments indicated that stomatal conductance and rate of transpiration were interdependent and it was interpreted to mean that stomatal conductance enhanced rate of transpiration at different times of the day. These plant extracts however caused an increase in the rate of transpiration of the bean plants, which resulted in loss of water. Results reveal bioactive potential of the flora from *M. volkensii* and a combination of *B. angustifolia* and *Z. chalybeum* to produce metabolites with potential applications as botanical pesticides.

Key words: Antifungal, beans, physiological responses, rust.

INTRODUCTION

The importance of the French beans is due to their high nutritive value in both energy and protein contents. Therefore, increasing the crop production is one of the most important targets of agricultural policy in several countries. The bean rust fungus (*Uromyces appendiculatus*) is of worldwide importance as a yield-reducing disease of *Phaseolus vulgaris* L., potentially cause yield losses up to 50% (Venette and Jones, 1982; Berger et al., 1995; De Jesus Junior et al., 2001). Under severe disease, it completely defoliates the plant and can cause 100% crop failure (Steadman et al., 2002). Rust result in harmful effects on growth, most physiological activities and the yield of beans. On global scale, studies have shown that some plant species have antifungal compounds (Fabry et al., 1996; Okemo et al., 2003). Within this context, natural products from plants seem to be a good alternative since numerous plants have the potential to control phytopathogenic fungi, and have much prospect to be used as a fungicide. Despite the many studies performed on biological control, relatively little is known about the role of the plant extracts (*Boscia angustifolia, Melea volkensii* and *Zanthoxylum chalybeum)* applied on the physiological parameters of the plants. In this study, we hypothesized that antifungal plant extracts might influence physiological activities of bean plants. Therefore, this study aimed at studying the role of selected plant extracts (added singly or in combination) in influencing photosynthetic activities of bean plants and finding an explanation for the above role based on test attributes.

MATERIALS AND METHODS

Study site

Field studies were carried out at 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 m above sea level and it receives an annual rainfall of 850 mm. Temperatures range from 13 and 26°C.

Collection and processing of plant materials

The samples of desired plants (*B. angustifolia, M. volkensii* and *Z. chalybeum)* from previous experiments (Omwenga, 2009; Kiswii, 2009) for 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 identified and verified 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 botany laboratory. The plant leaves and roots were dried separately at room temperature for a period of 1-2 weeks and then ground separately to powder using a grinding mill at 8000 rpm (Type 8 lab mill). The powder was stored in plastic bags at room temperature until the time required. Two kilograms of each plant sample was soaked and left overnight to allow extraction of the crude active compounds. The supernatant was filtered in several layers of muslin cloth and volumes adjusted to 20 L. (Stoll, 2000). A combination of *B. angustifolia, M. volkensii* and *Z. chalybeum* extracts was used because previous experiments (Menge, 2011) revealed a better synergism between the two in reducing the disease severity. A normal washing bar soap ground to powder and dried was used as a sticker at a rate of 1 g per litre of water extracts. Untreated control was used containing water and soap only without the extracts. During the growing period, beans were infected with rust from natural inoculum at the field. Seeds were obtained from Regina Seed Company and planted at a spacing of 30 cm between rows and 10 cm between plants within the rows (Monda et al., 2003). French bean seeds commercially available coated with thiram were used to control root rots. French bean variety Amy seeds were planted in 4 × 3 m plots each separated by a 1 m path between the treatments and the replications. Amy is high yielding as compared to other varieties therefore it is grown by most farmers. Di-ammonium phosphate was used at planting, at a rate of 200 kg/ha mixed well before seed placement. Calcium ammonium nitrate was applied at a rate of 100 kg/ha at trifoliate leaf stage.

Experimental design type

The experiment was carried out in a randomized complete design, and data analyzed using analysis of variance; and comparison of means was made by using Duncan's multiple range test. 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. A spray regime of once a week using a knap sack was employed from the fifteen days after planting until flowering. The extracts were used as protectants. The fungicide was applied at a rate of 2.5 kg ha^{-1} according to the manufacturers' recommendations. There were a total of seven hundred and sixty plants per replicate. Overhead irrigation twice a week and weeding were done as necessary.

CO_2 exchange measurements

Three different types of leaf gas exchange measurements were made on plants from the interior rows of the plots. First, at approximately weekly intervals, measurements of carbon dioxide assimilation rate were made at 0900, 1200, and 1500 h at the JKUAT farm. Mature, fully illuminated upper canopy leaves were measured at their nominal daytime growth. Daylight patterns of carbon dioxide assimilation rate were measured by the infrared gas analyzer (IRGA). IRGA was used as a null point instrument that allows the flow of carbon dioxide into the system at a rate equivalent to the rate of uptake of the leaf. The amount of carbon dioxide assimilated by the leaf was read directly from the IRGA. French bean leaf tissues from ten selected plants from each treatment were enclosed in the leaf chamber (leaf chamber = 2.5 cm^2) one at a time. The air flow rate through the chamber remained fixed. The carbon dioxide assimilation was monitored for 1 min for each leaf by the IRGA connected in an open gas flow system. During measurement of CO_2 assimilation rate the following parameters were also recorded using IRGA; stomatal conductance, and transpiration. IRGA determines the rates of photosynthesis (AN) and transpiration (E), as:

$$AN = (\text{Air flux} \times \Delta CO_2)/\text{Leaf area}$$

$$E = (\text{Air flux} \times \Delta H_2O)/\text{Leaf area}$$

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Abbreviations: PAR, Photosynthetic active radiation; **C_3,** carbon parameters; **JKUAT,** Jomo Kenyatta University of Agriculture and Technology; **Pn,** photosynthetic rate; **IRGA,** infrared gas analyzer; **E,** transpiration; **gs,** stomatal conductance; **Cu^{2+},** copper; **kg/ha,** kilograms per hectare.

Table 1. Selected antifungal plant extracts 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

From Fick's first law of diffusion, other parameters (stomatal conductance and CO_2 assimilation rate) were then calculated.

RESULTS

Stomata conductance (gs) and transpiration rate

The diurnal changes in gs, rate of transpiration and photosynthetic rate under the antifungal treatments are as shown in Figures 1a and b. The stomatal conductance in Figure 1a followed the same pattern in all the treatments being highest at 9:00 am, dropped at midday and maintained low levels in the late afternoon. However, there were significant differences in stomatal conductance (P=0.0173) in the treatments at 9:00 am and was rated as *B. angustifolia - Z. chalybeum* (77.7 mol/m^{-2}sec^{-1}) combination having the highest stomatal conductance followed by, *M. volkensii* (46.3 mol/m^{-2}s^{-1}) and Kocide DF (39.18 mol/m^{-2}s^{-1}), respectively. *M. volkensii* plant extract showed lowest stomatal conductance as compared to the other plant extract combination. The stomatal conductance for commercial fungicide (Kocide DF) was significantly lower (25.6 mol/m^{-2}s^{-1}) than other treatments at 12:00 pm. *B. angustifolia - Z. chalybeum* (41 mol/m^{-2}s^{-1}), *M. volkensii* (41.5 mol/m^{-2}s^{-1}) and untreated control (38.3 mol/m^{-2}s^{-1}) were not significantly different from each other at 12:00 pm. There were no significant differences in stomatal conductance at 15:00 pm of all treatments (P=0.1235). This showed that apart from controlling fungal attack the treatments had some influence on stomatal conductance. This behavior was observed in all the four treatments in the three months growth period. Figure 1b shows the diurnal changes in the rate of transpiration in the four treatments. The rate of transpiration was highest at 9:00 am coinciding with highest stomatal conductance and dropped at noon when stomatal conductance also dropped and maintained low levels in the early afternoon and evening when stomatal conductance and PAR were low. There were significant differences (P=0.003) in transpiration rates of the treatments at 9:00 am. *B. angustifolia - Z. chalybeum* (2.065 mol/m^2/s) and *M. volkensii* single plant extracts (1.353 mol/m^2/s) had significantly the highest rate of transpiration as compared to other treatments. There were significant differences (P=0.0015) in the rates of transpiration among the treatments at 12:00 pm. Kocide DF (0.76 mol/m^2/s) had significantly the lowest rate of transpiration while there were no differences in *B. angustifolia - Z. chalybeum* (1.12 mol/m^2/s), *M. volkensii* (1.135 mol/m^2/s) and untreated control (1.067 mol/m^2/s). There were significant differences (P<0.05) in transpiration rates of the treatments at 15:00 am. *B. angustifolia - Z. chalybeum* (0.67 mol/m^2/s), *M. volkensii* (0.78 mol/m^2/s) and Kocide DF (0.77 mol/m^2/s) treated beans had lower rates of transpiration at 15:00 pm than the untreated control (1.3 mol/m^2/s) at 15:00 pm. Generally both the single and combinations of plant extracts had a positive effect on the rate of transpiration. The high positive regressions (r^2 >0.9) and the regression equations are summarized in Table 2.

Effect of treatment on CO_2 assimilation and photosynthetic rate (Pn)

Figures 1 and 2 show diurnal changes in photosynthetic (Pn) and CO_2 assimilation rates in the four treatments, respectively. The more the negative CO_2 assimilation the more CO_2 is absorbed from the environment as shown in Figure 2. The CO_2 assimilation reached a peak at 9:00 am and decreased sharply at noon and eventually maintained low levels in the afternoon. CO_2 assimilation followed the same pattern as that of stomatal conductance. There were significant differences (P<0.001) in CO_2 assimilation rates among treatments at 9:00 am. *B. angustifolia - Z. chalybeum* (577.933 ppm) treated bean plants had significantly lowest CO_2 assimilation rate while there were no differences between *M. volkensii* (679.5 ppm), Kocide DF (641.364 ppm) and untreated control (651.154 ppm) in CO_2 assimilation rate at 9:00 am. There were no significant differences (P>0.002) in CO_2 assimilation rate of all treatments at 12:00 pm however they ranged from untreated control (362 ppm) being the highest then followed by *B. angustifolia - Z. chalybeum* (328.33 ppm), *M. volkensii* (320.33 ppm) and Kocide DF (304.18 ppm), respectively. Likewise, at 15:00 pm there were no differences (P=0.1425) in CO_2 assimilation rates of all treatments. The relationship between stomatal conductance and CO_2 assimilation was described by low insignificant positive regressions in each treatment as shown in Table 3. The low R^2 indicated the two parameters were very slightly interrelated. The diurnal pattern of rate of photosynthesis among the treatments was the same being highest at the morning, 9:00 am dropped at noon and remained low in the afternoon (15:00 pm). There were significant

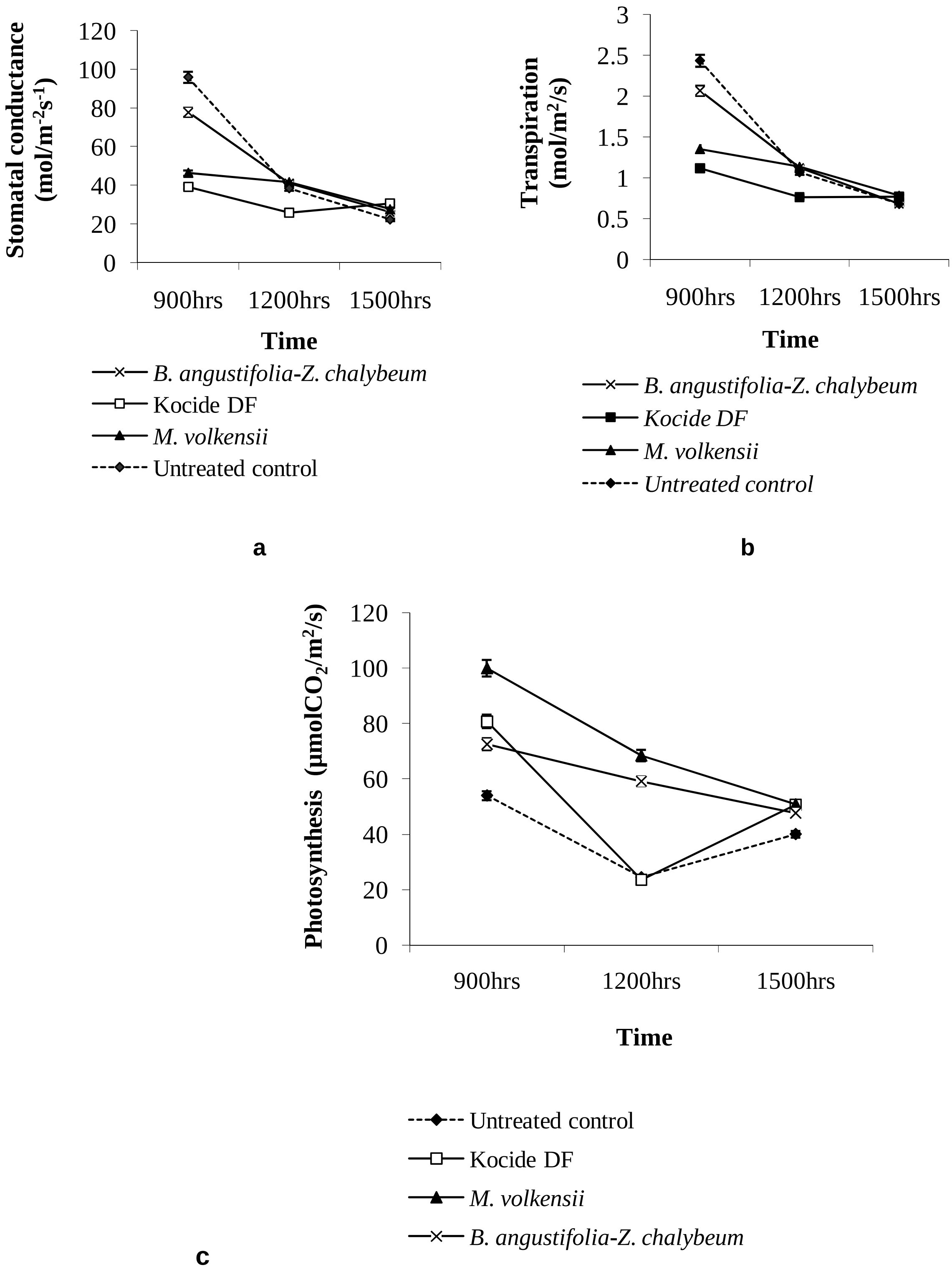

Figure 1. Daily diurnal courses of stomatal conductance (a), rate of transpiration (b) in French beans (Amy variety) exposed to various treatments. Each point represents the mean ± standard error of six replications.

differences ($P=0.0021$) in the rate of photosynthesis among the treatments at 9:00 am. The rate of photosynthesis was rated highest in *M. volkensii* (99.9 μmolCO_2/m^2/s) as compared to the combination *B. angustifolia - Z. chalybeum* (72.5 μmolCO_2/m^2/s) and untreated control (53.9 μmolCO_2/m2/s), respectively.

Table 2. The relationship between stomatal conductance and rate of transpiration among the four treatments.

Treatment	Equation	R^2
B. angustifolia - Z. chalybeum	y =44.851x -10.454	0.9829
Untreated control	y = 41.604x -4.882	0.9656
Kocide DF	y = 38.824x -3.8036	0.9449
M. volkensii	y = 37.407x -0.7395	0.9396

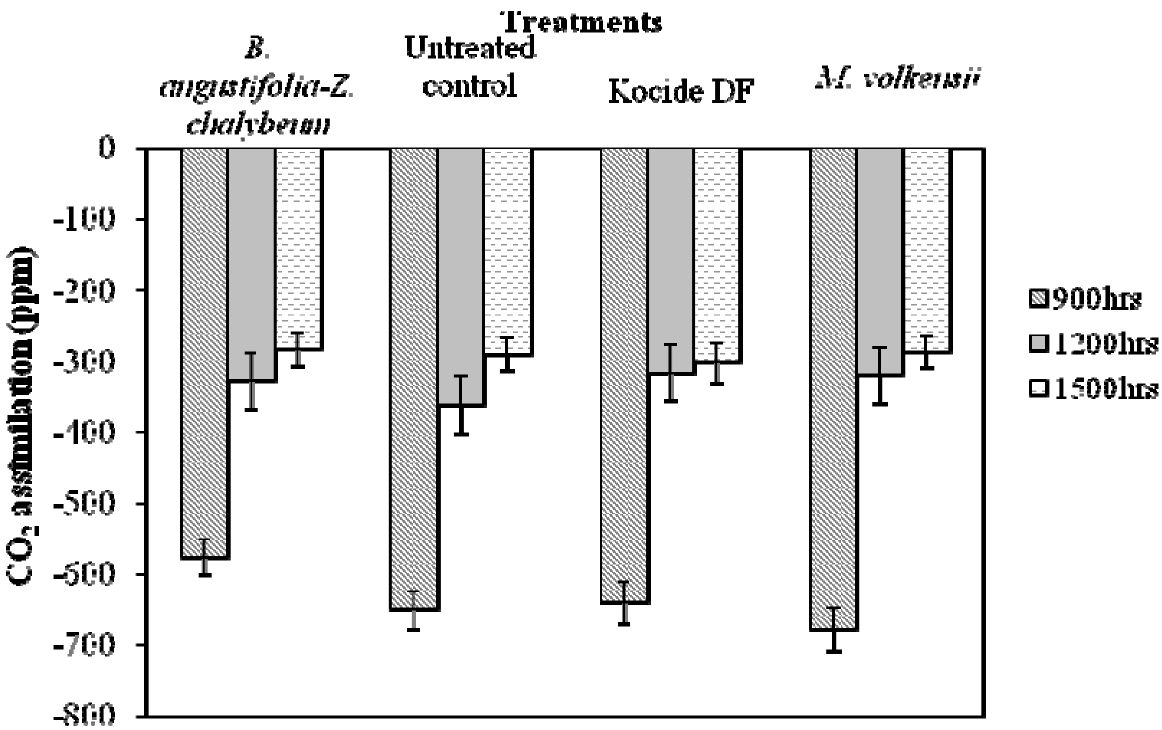

Figure 2. Daily courses of CO_2 assimilation in French beans exposed to various antifungal plant extracts and a commercial fungicide under natural conditions.

Table 3. Linear relationships between CO_2 and the rate of photosynthesis.

Treatment	Equation	R^2
B. angustifolia - Z. chalybeum	y = 4.0963x + 74.228	0.5873
Untreated control	y = 0.0369x + 39.852	0.0801
Kocide DF	y = 0.1134x + 7.1438	0.4250
M. volkensii	y = 0.0994x + 27.677	0.6596

There were significant differences (P=0.0132) in the rate of photosynthesis among the treatments at 12:00 pm. *M. volkensii* (68.38 $\mu molCO_2/m^2/s$) had significantly the highest photosynthetic rate at 12:00 pm followed by *B. angustifolia - Z. chalybeum* (59.1 $\mu molCO_2/m^2/s$). However, there were no differences between Kocide DF (23.51$\mu molCO_2/m^2/s$) and untreated control (24.4 $\mu molCO_2/m^2/s$) at 12:00pm. *M. volkensii* (50.77 $\mu molCO_2/m^2/s$) and Kocide DF (50.7 $\mu molCO_2/m^2/s$) revealed significantly the highest rates of photosynthesis although they were not different from each other at 15:00bpm. Untreated control (39.98 $\mu molCO_2/m^2/s$) had the lowest photosynthetic as compared to other treatments at 15:00 pm. Generally, *M. volkensii* had a positive effect on rate of photosynthesis as compared to other *B. angustifolia - Z. chalybeum.*

DISCUSSION

Effect of treatment on some selected physiological parameters

Generally, French bean leaves showed higher values of stomatal conductance with consequent higher transpiration. The high positive regressions (r^2 >0.9) were obtained in the four treatments. This indicated that stomatal conductance and rate of transpiration were interdependent and it is interpreted to mean that stomatal conductance enhanced rate of transpiration at different times of the day.

The response of stomata to transpiration was used by Monteith (1995a), who re-analyzed 52 sets of published measurements at canopy scale of humidity responses on 16 species of monocots in terms of the relation between stomatal conductance and transpiration. *B. angustifolia* - *Z. chalybeum* combination and single plant treatment *M. volkensii* had a positive effect on stomatal conductance of bean plants. This suggests that these antifungal plant extracts in general may have interfered with any one of the several biosynthetic pathways or energy production pathways. Commercial control (Kocide DF) had the lowest stomatal conductance of all treatments however; Kocide DF plots had the lowest water loss as compared to others, this indicates they were better at water conservation. Stomata showed a slight opening tendency until 1200 noon, when decreases in stomatal conductance were likely cut down in high transpiration (E) values. Since similar stomatal conductance values were observed during morning, changes in E values suggest that stomatal aperture was more than sufficient to support maximal E values since early hours of morning.

The high regressions between stomatal conductance and rate of transpiration in the four treatments indicated that stomatal conductance and rate of transpiration were interdependent and it was interpreted to mean that stomatal conductance enhanced rate of transpiration at different times of the day. The differences in R^2 values in four treatments were insignificant meaning that concerning these two parameters, the French beans responded to the treatments the same way. This pattern was maintained throughout the growing period. Therefore, the sources of variation in stomatal conductance and the rate of transpiration were treatment, time.

The daily diurnal courses conformed to Zeiger et al. (1981) study which showed that at dawn, stomatal conductance usually increases very rapidly because the entrained rhythm is in correct phase, and also there is a great sensitivity to low photon fluxes of blue light at this time. Stomatal conductance then increases gradually towards a maximum value in late morning or early afternoon before declining noticeably later in the day. This partial closure in the afternoon is thought to be driven by the entrained rhythm, and it is not unusual for the stomata to be nearly closed before dusk. The responsiveness of stomata to light and CO_2 depends on leaf age and past treatment. As leaves become older, the stomata often become less responsive and may open partly, even at midday. It is difficult to generalize stomatal behavior because so many contradictory reports occur in the literature. Stomatal activity is affected by numerous internal and external factors which often interact in complex ways that sometimes are overlooked by investigators.

Daily course of CO_2 assimilation was similar for all evaluated treatments. In early morning, the sharp increase in photosynthetic photon flux density seems to be the main cause of CO_2 assimilation increase. Considering the highest CO_2 assimilation values, no significant difference was found between treatments under natural condition. Maximal CO_2 assimilation rates were reached around 9.00 am until 12:00 pm when reductions were recorded. Low stomatal conductance is known to cause decrease in CO_2 assimilation values by reducing the CO_2 available, which may be indicated by decreased intercellular CO_2 concentration values (Jones, 1998; Nobel, 1999). Commercial control (Kocide DF) treated plants had the lowest carbon dioxide released as compared to all other treatments because of its low stomata conductance.

Photosynthetic rates (Pn) among the four treatments followed a trend whereby they were at the peak at 9:00 am reducing gradually towards the afternoon and at 15:00 pm. The main sources of variation in the Pn might have been due to treatment and the time of the day. The explanation for the above stated interactions being significant could be that these factors were affecting the photosynthesis rates dependently. The antifungal plant extract had a positive effect on the rate of photosynthesis than other treatments. Therefore, the results suggest that the photosynthetic capacity of ‘commercial control (Kocide DF) treated beans’ were constrained at natural condition by low stomata conductance. Low stomata conductance in the commercial control (Kocide DF) treated plants might have affected the photosynthetic activity. The inactivation of Rubisco (ribulose-bisphosphate carboxylase/oxygenase) a key-enzyme of Calvin cycle and its two accompanying enzymes, that is, Rubisco activase and carbonic anhydrase under the stress conditions caused by copper and lead (not examined) may be regarded as another possible factor (Vojtechova and Leblova, 1991). This indicated that plant extract treatments were leaf physiology friendly as compared to the copper containing Kocide DF. The untreated control highest transpiration rates might have been caused by high disease severity. Rust caused increased transpiration (E) from infected tissues after sporulation in untreated control. Transpiration before sporulation, which potentially is by a mainly stomatal pathway, is inhibited, probably by stomatal closure; rust is known to inhibit stomatal opening in the light in other diseases, e.g. bean (*P. vulgaris*) infected by either *U. phaseoli* (Duniway and Durbin, 1971b) or *Uromyces appendiculatus* and this effect has recently been confirmed for Faba bean rust (Tissera, unpublished results). In the present study, it was noted that at each sample time, more variability in transpiration rate occurred in rusted tissue than in healthy tissue. This variability probably occurred because the number of lesions per unit area of leaf was not controlled. Durbin (1978) stated that when sporulation occurred, transpiration from bean leaves infected with rust increased by as much as 50%. Where net photosynthesis was concerned, infection induced opposing changes in the four treatments; net photosynthesis in healthy leaves increased because gross photosynthesis was stimulated and photorespiration was inhibited. Net photosynthesis per plant and ultimately plant growth of the untreated control reduced because infection inhibits the growth of leaf area.

Photosynthesis is closely related to crop growth and yield, and higher photosynthetic rate of leaves is one of the important factors for high crop yield. The results showed that after flowering, the leaves gradually aged, the net Pn, E and gs of leaves gradually declined. Commercial control (Kocide DF) contains copper metal that might have caused low productivity. This could be attributed to its contents that can hamper the process of photosynthesis. It being a micronutrient, copper improves plant growth at natural concentrations. However, at higher concentrations, it also proves very toxic to plants. The phytotoxic effects related to higher concentrations of copper include inhibition of photosynthetic efficiency and as a result reduced crop productivity. The process of photosynthesis was adversely affected by Cu toxicity. Plants exposed to copper formulated fungicide (Kocide DF) showed a decline in photosynthetic rate, which might have resulted from distorted chloroplast structure, restrained photosynthesis of chlorophyll and carotenoids, inhibited activities of Calvin cycle enzymes, as well as deficiency of CO_2 as a result of stomatal closure (Vojtechova and Leblova, 1991).

A strong relationship exists between Kocide DF application and a decrease in photosynthesis and it is believed to result from stomatal closure. Increased rates of respiration and loss of chlorophyll from the leaf tissue apparently were the major factors responsible for the reduction of photosynthetic rates on diseased untreated control leaves.

The photoinhibition mechanism could have a character of photoprotection or represent damaging in PSII reaction centers (Osmond, 1994). The maximum CO_2 assimilation values observed are in agreement with the measurements performed by Souza et al. (2003) in common bean study. Transpiration exhibited similar trend to photosynthesis suggesting that an appreciable part of the inhibition of the two processes is related to increased stomatal resistance as a result of stomatal closure.

Conclusion

B. angustifolia - Z. chalybeum combination and single plant treatmentof *M. volkensii* had positive effects on enhancing the rate of photosynthesis in bean plants. The high regressions between stomatal conductance and rate of transpiration in the treatments indicated that stomatal conductance and rate of transpiration were interdependent and it was interpreted to mean that stomatal conductance enhanced rate of transpiration at different times of the day. These plant extracts however caused an increase in the rate of transpiration of the bean plants, which resulted in loss of water.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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