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Research Article

Effects of Benomyl Soil Treatments on Cassava Response to Diseases, Pests and Mycorrhizal Symbiosis

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ABSTRACT

This paper studied the effects of benomyl soil treatments on cassava response to diseases, pests and mycorrhizal symbiosis. It highlights the importance of the use of benomyl- a fungicide, to control incidences of pests and diseases of cassava for sustainable agriculture. Soil drenches of benomyl (Methyl-I-butylcarbomyl)–2-benzimidazolecarbamate) were applied at 0, 25, 100, 200, 500 and 1000 µg/g in 4.2 kg heat sterilized soil samples. Mycorrhizal spores in soils and root colonization of cassava were estimated at 5.5 months after planting. Shoot heights and girths (cm) and leaf numbers were assessed at 5.5 months. Incidences of cassava green spider mite (CGM) and cassava mealy bug (CM)were rated on a scale of 1 (symptom free) to 5 (severe symptom); African cassava mosaic disease (ACMD) rated bimonthly from 3.5 to 5.5 months on a scale of 0-5; cercospora leaf spot disease (CLSD) assessed on the leaf spot numbers per plant and the infection index calculated. Plants grown in 200, 500and 1000 µg/g benomyl treated soils died of severe phytotoxicity. Mycorrhizal spores and root colonization were significantly higher in the control expe riments ($P<0.01$) with percentage decreases of 44 and 67% per g/soil of spores, and root colonization of 31 and 48%, in the 25 and 100 µg/g, respectively. Benomyl treatment at 25 and 100 µg/g, respectively, significantly increased plant vigour (stem and girth) ($P<0.01$), with increases by 12.15 to 18.69% for height, and 8.47 to 23.73% for girth. Mycorrhizal symbiosis was lowest in plants amended with doses of benomyl. Effects of benomyl significantly reduced CLSD ($P<0.05$). No significant effects were detected for CGM, CM and ACMD. The study showed effects of benomyl in increased plant growth and capable of controlling a viral plant disease (CLSD) of cassava.

Key words: benomyl, Mycorrhizal, cercospora leaf spot disease (CLSD), African cassava mosaic disease (ACMD).

INTRODUCTION

Poor management practices in the use of pesticides in agriculture often result in many problems in the environment such as water, soil, animals and food contamination [1]. The impact of benomyl, a systemic fungicide is prominent in the rhizosphere where it may eliminate and benefit some microorganisms. Its effects are also crucial in delayed senescence cow pea (*Vigna unguiculata* (L) Walp)[2]

Arbusculasr mycorrhizas (AM) are symbiotic fungi that form symbiotic and non-pathogenic association with soil borne fungi and roots of higher plants [3];[4]. The implication of this association is that AM fungi are greatly involved in nutrient uptake such as P, in virtually all agricultural and native plants. [5]; [6]; [7]. Nutrient transfer is attributed to plants' dependency on AM root colonization and spores in soils. Among plants that are obligate mycorrhizal is cassava (*Manihot esculenta* Crantz) which can grow in infertile and impoverished nutrient deficient soils (especially P), zinc and copper [8]; [9]. Resident infective propagules of AM are chlamydospores, infected root pieces, infective structures in living roots and clumps of hyphae, contribute to nutrient mobilization [10].High throughput genetic analyses parallel to gene expression, protein and metabolic profiling have literarily changed our view of the arbuscular mycorrhizal symbiosis [11]; [12]. These methods have helped to unravel many aspects of the AM physiology. The physiological activity of AM is affected by benomyl. Benomyl is known to profoundly inhibit and decimate mycorrhizal functions, structures and those of other fungal pathogens [13]; [14]; [15].

Cassava is afflicted with many pests and diseases, including the more serious ones such as Cassava green spider mite (CGM), Cassava mealy bug (CM), African cassava mosaic disease (ACMD), Cercospora leaf spot disease (CLSD) and cassava bacterial blight (CBB) among others. The pests feed on leaves of cassava, thereby causing damage, defoliation and yield losses [13]. Incidence of leaf pathogens greatly diminishes the photosynthetic capacity of cassava, which can lead to lower infections by fungal endophytes [13].

Fungicides remain a vital solution to the effective control of plant diseases which are eliminated to cause yield losses of almost 20% in major food and cash crops worldwide [16].There appears to be lack of comprehensive reports on the effects of benomyl fungicide on cassava response to incidences of pests and diseases, and mycorrhizal symbiosis. This paper therefore highlights the importance of the use of benomyl- a fungicide, to control incidences of pests and diseases of cassava for sustainable agriculture.

MATERIALS AND METHODS

Fungicide Treatment of Soil Sample

Samples of a sandy loam garden top soil were collected from a field plot at the University of Benin, Benin City and were dispensed in medium-size (18 x 36 cm)polyethylene bags (poly bags). The soil was heat sterilized[13].Batches of the soil samples in the poly bags were treated with a systemic fungicide, benomyl (Methyl-I-(butylcarbamoyl)-2-benzimidazole carbamate) at 25, 100,200, 500, and 1000 µg/g active ingredient (a.i). For application of powdered fungicide formulation which contained 50% (a.i) was weighed in 0.10, 0.39, 1.97,and 3.95 g and added/mixed to each soil sample (4.2 kg)in poly bags. Untreated soil samples served as control. Benomyl was applied to the soil samples as drenches by dissolving each benomyl sample in 300 ml of distilled water and thereafter soaking the soil samples with each of the fungicide solutions. Control soil samples were drenched only with 300 ml of distilled water..The fungicide treated soil samples were placed in each plastic bowls (receptacles) and stationed on a raised platform outdoors for about six months period of study.

Cassava Seedlings in Open-Sided Shed Screen House

Two node stem-cuttings of about 10 cm of a cassava (*Manihot eculenta* Crantz) clone (TMS 30555) were sprouted for two weeks in saw dust soaked daily, with tap water in 30 x 30 cm wooden boxes. Each plant was transplanted into benomyl treated and un treated soil samples. The plants were watered on alternate days for14 days by adding 200 ml of tap water to the soil in each bag, and there after transferred to a roofed open-sided screen house. The potted plants were arranged according to treatment rates and completely randomized.

Mycorrhizal spore content and degree of root colonization estimates of mycorrhizal spores in each soil samples were assessed. Quantification of spores was carried out after soil samples were processed for spore recovering using the technique of wet sieving and decantation [17]. The numbers of mycorrhizal spores extracted by this technique were estimated using a calibrated stereomicroscope micrometer slide. Three replicate samples of mycorrhizal spores in Petri plates were evaluated and their means determined. The degree of AM root colonization was assessed by the method of [18]. Fine feeder roots of 5.5months-old cassava test plants were bulked, washed and cut into 1-cm, and stored in vials containing formal-acetic alcohol(FAA) as a fixative. The root pieces processed were stained with 0.05% Trypan blue in lactophenol. The root pieces were then randomly selected and mounted on microscope slides in group of 10, and there after examined and scored for the degree of root colonization under a binocular microscope following the method of [19].

Pest infestation scores for cassava green spider mite and cassava mealy bug were evaluated, for cassava green spider mite (*Mononychellustanajoa* Bonder) and cassava mealybug (*Phenacoccus manihoti* Matt. Ferr.), infestation symptoms were evaluated on a scale of increasing symptom severity, from I (symptom free) to 5 (severe symptoms) with intermediates [20]; these symptoms were rated from 3.5 - 5.5 months for cassava green spider mite, while those for cassava mealy bug were rated from 5 - 5.5 months. Disease infection ratings for African cassava mosaic virus and *Cercospora* leaf spot disease were carried as follows. Incidence of African cassava mosaic virus symptoms on leaves of cassava was scored from 3.5 - 5.5 months on a scale of 0 - 5 where 0 is no symptom and 5 severe symptoms with intermediates [21]. Visual assessment of *Cercospora* leaf spot disease.*Cercospora* leaf spot disease assessment was based on the number of leaf spots per plant [13]. Disease intensity was determined by calculating the infection index [22].

Assessment of Shoot height and girth (vigour) and leaf numbers at 5.5 months shoot height and girth of cassava were measured using a field measuring tape, and a venier caliper, respectively. Leaf numbers were obtained by visual count of leaves per plant in soil samples amended with and without benomyl Mycorrhizal Symbiosis (MS) of cassava Mycorrhizal symbiosis or dependency for height and girth measurements of test plants was calculated as the degree to which treated plants depended on AM to grow, i.e., height or girth data of control and treated plants as a percentage of control plant data[23].

RESULTS AND DISCUSSION

Effects of benomyl on AM spores and root colonization were obtained. Cassava seedlings grown in soils amended with 200,500, and 1000 µg benomyl/g soil died from severe phytotoxicity. Therefore, data of spore count and root colonization were taken from the control, 25 and 100 µg benomyl/g soil of 5.5 months old cassava. Control soils consistently had more spores than those receiving benomyl (Table 1).

Table1: Arbuscularmycorrhizal spores/g soil and root colonization of cassava seedlings as affected by benomyl

Benomyl (µg/g)	Spore/g Soil	Root Colonization (%)	Decreases (%)	
			Spore	colonization
0	0.09a	73.33	-	-
25	0.05	51.33	44	31
100	0.03	38.67	67	48
P<0.05				

[a] means of three replications; **–** replicate values of spore number in 100 g of soil at treatment concentration

Increasing levels of benomyl titresdepressed mycorrhizal spores (P<0.05). Percentage decreases in AM spores were 44 and 67% for 25 and 100µgbenomyl, respectively, (Table 1). Similar findings showed suppressive effects of benomyl [24];[13]2005). This is attributed to the decomposition product of benomyl (benzimidazole-2-ylcarbamate) that suppresses the germination of chlamydospores [25].Percentage root segment colonization ratings were highest among roots of plants in the control soils and lowest in roots of plants treated with 100 µg/g the highest benomyl concentration (P<0.01) and decreases recorded were 31 and 48% for 25 and 100 µg/g soil treatment, respectively (Table 1). Results obtained are inconformity with similar results on the depressive effect of benomyl on root colonization by AM [26];[14];[27].

Symptoms of cassava green spider mite were marked by large numbers of small yellow pin-prink spots on leaves which were reduced in area, distorted, and turned rough and mosaic yellow. Observed trends showed no significant effect of benomyl concentrations on the reduction of cassava green spider mite symptoms whencompared with control stands (Table 2). Cassava mealybug attacked young and matured leaves. The symptoms included mealy bug signs on the undersurface. No effect of benomyl on cassava mealy bug was apparent. However, cassava mealy bug incidence was generally reduced (Table 2). There is no information available on the effect of benomyl on cassava green spider mite and cassava mealy bug incidence, and this requires further investigation.

Effect of benomyl on African cassava mosaic virus, Cercospora leaf spot disease were obtained. Symptoms of African cassava mosaic virus appeared as growth abnormalities, include d severe mosaic patterns and distortion of leaflets. No discernable trend in mosaic incidence with benomyl concentrations was apparent. Incidence of African cassava mosaic virus was generally low (Table 2).The use of benomyl against African cassava mosaic virus infection proved to be ineffective.[28]pointed out that chemical control of virus is futile due to the population dynamics and ubiquity of the white fly vector.Cercospora leaf spot symptoms developed at the bottom leaves or older leaves. The spots were roughly circular, gray to brown, ca 9-10 mm in diameter. In general, cercospora leaf spot disease indexes from control plants were higher than values from plants grown in soil with benomyl increasing titres (Table2). Benomyl decreased the incidence ratings generally(P<0.05).

Table 2: Effects of benomyltitres on the incidences of CGM, CM, ACMD and CLSD ratings on 3.5 - 5.5 months cassava seedlings

Benomyltitre	Pest incidence rating		Disease incidence rating	
(µg/g)	CGM	CM.	ACMD	CLSD
0	2.36b	2.04	1.72	8.81
25	2.16	1.53	1.53	4.86
100	2.11	1.85	1.85	3.76
P<0.05				

[a] are ratings of incidences of pests and diseases; [b] means of four replications.

The reduction of cercospora leaf spot disease infection by benomyl is ascribed to the innate toxicity ofthe fungicide which moves systemically in plants. The possibility of the effect of benomyl on disease development recorded in this study agrees with findings of [25].

Effect of benomyl on plant vigour and leaf numbers- Height and girth data were highest in plants from soils amended with benomyl, while the lowest values were from plants in soils without benomyl application. General increases were related to increasing benomyl concentrations. Mean heights from plants in soils with 25, and 100 µg/g benomyl increased 12.15 and18.69%, respectively, and similarly girth 8.47 and 23.73%, respectively, over control (Table 3).Leaf numbers were highest on plants in soil treated with 100 µg/g benomyl and least in control plants (Table 3). Similar reports of the positive effect of benomyl in improving plant vigour have been documented [29].

Effect of benomyl on mycorrhizal symbiosis (MS)- Mycorrhizal symbiosis for height and girth of test plants was affected by benomyl treatment. Lowest values of MS were in plants in soils with 100 and 25 µg/g benomyl and highest in control plants (Table 3). This report on the depressive effect of benomyl on MS agrees with findings of [30].However, the present studyrevealed unexpected increases in height and girth of plants despite the depressive effect of benomyl.

Table 3. Effects of Benomyl titres on the Height, Girth, Leaf number and Mycorrhizal Symbiosis of cassava seedlings

Benomyl µg/g	Actual data		Increases (%)		MS(%)		Leaf number (cm)
	Height (cm)	Girth (cm)	Height (cm)	Girth (cm)	Height (cm)	Girth (cm)	
0	26.75a	0.59a	-	-	15.75	19.18b	11
25	30.00a	0.64a	12.15	8.47	5.51	12.33	11.86
100	31.75a	0.73a	18.69	23.73	0	0	14.96

a-means followed by the same letter in each column are not significantly different at 5% level; **b**-means of five replications.

CONCLUSION

In Nigeria and most parts of the Third World, agricultural sustainability is a major challenge mainly attributable to disease and pest incidences, and lack of storage facilities. The impact of hunger and its attendant malnutrition especially in children is a glaring threat to life. Benomyl is a system fungicide that is selectively toxic to microorganisms. It acts against a wide range of fungal diseases of arable and vegetable crops [31].Benomyl is a broad spectrum fungicide that is active against the Ascomycetes, Basidiomycetes and some Deuteromycetes.Benomyl binds strongly to soil and does not dissolve in water to any considerable extent and is readily absorbed through the root system of the plants. The import of this study highlights the use of benomyl fungicide to control diseases and pests of agricultural crops when the fungicide is applied at moderate doses, avoiding high dosage applications that can lead to severe phytotoxicity and death of plants. Benomyl therefore, significantly increase crop yields and plant vigour for greater agricultural sustainability.

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