African Crop Science Journal, Vol. 13. No. 3, pp. 209-214, 2005
Printed in Uganda. All rights reserved
ISSN 1021-9730/2005 $ 4.00

SHORT COMMUNICATION

EFFICACY OF SYNTHETIC AND NON-SYNTHETIC FUNGICIDES AGAINST STEM CANKER DISEASE OF TEA

M.O. ONIANG'O, K.B. KHARE* O. S. ACHWANYA and W. OTIENO[1]
Department of Botany, Egerton University, Njoro-536, Kenya
[1]Tea Research Foundation of Kenya (TRFK), Kericho, Kenya

(Received 8 January, 2004; accepted 12 September, 2005)

ABSTRACT

Tea *(Camellia sinensis)* is a major source of foreign exchange in Kenya. Its production is greatly affected by the stem canker disease *(Phomopsis theae)* and, thus, the need to develop effective control strategies. The efficacy of three fungicides (copper oxychloride, mancozeb and benomyl at concentrations of 10, 25, 50, 100 ppm for each, and acqueous leaf extracts (10% w/v) of four indigenous plants, sage brush *(Lippia javanicum),* stinging nettle *(Urtica massaica),* savory *(Satureia biflora)* and Kenya green heart *(Warburgia ugandensis)* were evaluated *in vitro* against two isolates (P 228 and P 794) of *Phomopsis theae* Petch, the cause of stem canker disease of tea. The variables used for the study of growth inhibition were colony diameter and number of pycnidia produced on malt extract agar, and dry weight of thallus biomass in liquid malt extract. The three fungicides differed significantly ($P \leq 0.01$) from each at all concentrations. Benomyl was the most effective as it completely inhibited the two isolates of *P. theae* in all concentrations. Mancozeb was the next best but it was effective only at high concentrations. Copper oxychoride was found least effective even at high concentrations. Aqueous leaf extract of *W. ugandensis* was the most effective and completely inhibited mycelial growth, biomass accumulation and pycnidial production even at very low concentrations.

Key Words: *Camellia sinensis*, fungicides, mycelial growth, *Phomopsis theae*, plant extracts

RÉSUMÉ

Le thé *(Camellia sinensis)* est une source majeure de devises au Kenya. Sa production est grandement affectée par la maladie canker de tige *(Phomopsis theae)* et, ainsi, le besoin de développer des stratégies effectives de contrôle. L'efficacité des trois fongicides (oxychlorure de cuivre, mancozeb et benomyle aux concentrations de 10, 25,50, 100 ppm de chacun, et les extraits liquide de feuille (10% w/v) de quatre plantes indigènes, sauge de broussaille *(Lippia javanicum)*, ortie dard *(Urtica massaica)*, savory *(Satureia biflora)* et Kenya green heart (Warburgia ugandensis) étaient évaluées in vitro contre deux isolé (P 228 et P 794) de *Phomopsis theae* Petch, la cause de la maladie de canker de tige de thé. Les variables utilisées pour l'étude de l'inhibition de croissance étaient le diamètre de colonie et le nombre de pycnide produit sur l'extrait d'agar de malt, et le poids au sec de la biomasse de thallus dans l'extrait liquide de malt. Les trois fongicides ont différé de façon significative (P=0,01) à partir de chacune des concentrations. Mancozeb était le deuxième meilleur mais il était effectif seulement aux concentrations élevées. Oxychlorure de cuivre était trouvé moins effectif même aux concentrations élevées. L'extrait de liquide de feuille de *W. ugandensis* était le plus effectif et avait complètement inhibé la croissance de mycélien, l'accumulation de biomasse et la production de pycnide même aux très faibles concentrations.

Mots Clés: *Camellia sinensis*, fongicides, croissance de mycélien, *Phomopsis theae*, extraits de plante

INTRODUCTION

Tea (*Camellia sinensis* (L.) Kuntze) is one of the main sources of foreign revenues in Kenya, a leading exporter of black tea contributing over 20% to the world tea market (Anon., 2001). Fungal diseases are some of the major constraints to tea production especially affecting the quantity. Amongst them is stem canker disease caused by *Phomopsis theae* Petch. This disease, first reported in Kericho and Kisii districts in Western Rift Valley, was initially considered sporadic in occurrence and of little importance (De Lima *et al.*, 1978). However, later Onsando (1988) reported increasing incidences of stem canker in Kisii. The disease is now widespread and considerably destructive (Otieno, 2001). It occurs on certain tea clones in wet weather in various farms of Kisii, Kericho and Nandi districts of Kenya (Otieno, 1998 & 2001). The disease causes death of tea bushes of two to eight years old after completely girdling the collar, therefore, substantially reducing tea production. However, losses in terms of quantity caused by the disease have not been quantified but seems to be substantial (Otieno, 1998). Currently, control strategies to control the disease are limited. The most commonly used are conventional preventive measures aimed at reducing the inoculum potential (Shanmuganathan and Rodrigo, 1966; Shanmuganathan and De Silva, 1968; Willat, 1970; Fordham, 1971; Haster, 1973; Otieno, 1998). There is scanty literature on fungicide use against stem canker disease in tea and the efficacy of most fungicides against the disease is uncertain. For instance, Shanmuganathan and Bopearatchy (1972) reported the effect of benomyl against *P. theae* under *in vitro* conditions. There are no recent studies on this and other fungicides. The present paper reports our study on the effect of some fungicides and aqueous leaf extracts of some indigenous plants on the growth of *P. theae, in vitro*. The implication of our results may provide a framework for future work in formulating management strategies for the disease.

MATERIALS AND METHODS

Two isolates of *P. theae,* P228 and P794 used in the present work were obtained from the Tea Research Foundation of Kenya (TRFK). Cultures were maintained on PDA at 4° C and subcultured every after two months.

Evaluation of fungicides. Five grams of malt extract agar (MEA) was dissolved in 100 ml sterilized distilled water in 13, 250 ml Pyrex conical flasks and sterilized under pressure at 121° C for 20 minutes. The fungicides were incorporated into the medium aseptically at 0.002 g, 0.005 g, 0.01 g and 0.02 g for copper oxychloride (50% WP), benomyl (Benlate^R 50% WP) and 0.0013 g, 0.0031g, 0.0063 g and 0.0125 g for mancozeb (Dithane M-45^R 80% WP) at 60° C to make 10, 25, 50 and 100 ppm (active ingredients), respectively. The fungicide-medium mixture was agitated thoroughly and 20 ml of the mixture dispensed into each 9-cm diam sterile disposable plastic petri-plates in a lamina flow hood. The MEA plates without any fungicide served as control. For assay on biomass accumulation in liquid medium, 18 g of malt extract was weighed separately and dissolved in 13, 1000 ml Pyrex conical flasks containing 600 ml of sterile water. Then, 0.012 g, 0.03 g, 0.06 g and 0.12 g of copper oxychloride and benomyl, and 0.0078 g, 0.0195 g, 0.039 g and 0.078 g of manozeb were added to each of the twelve flasks to make 10, 25, 50 and 100 ppm, respectively. The liquid ME in conical flasks without any fungicide served as a control. One hundred and fifty ml of each fungicide – liquid ME was dispensed into 250 ml conical flask. Each conical flask with mixture was agitated thoroughly using a magnetic stirrer hot plate and sterilised under pressure at 121° C for 20 minutes.

Both MEA plates and conical flasks with liquid ME containing fungicides were inoculated aseptically in a lamina flow hood with 2 mm mycelial discs cut from the margin of three days old cultures of *P. theae* and incubated at 20±2° C. The radial mycelial growth of the colony on MEA (mm) and pycnidial production were determined after 8 days and 14 days of inoculation for isolate P228 but after only 8 days for isolate P794, a fast growing isolate that covers 9 cm plate completely in 8-9 days after inoculation. Pycnidial production on MEA plates was determined by counting them visually and were rated in each experiment as: abundant ++++ (above 50 pycnidia); good +++ (30-50 pycnidia); moderate ++ (15-30 pycnidia);

scanty + (below 15 pycnidia); nil - (No pycnidium). The fungal bio-mass of the two isolates on liquid ME was harvested 21 days after inoculation by filtering on Whatman's filter paper (No. 1) previously dried to constant weight. The harvested biomass with the filter paper was dried at 60° C for 48 hours and dry weight (g) in each treatment was determined. All the treatments were replicated three times and the data were subjected to analysis of variance to test for differences between effects (Steele *et al.*, 1997)

Evaluation of fungicidal properties of indigenous plants. Fifteen grams fresh leaves, each of sage brush (*Lippia javanicum* (Burm.) Spreng), stinging nettle (*Urtica massaica* Mildbr), savory (*Satureia biflora* (Don) Benth.) and Kenya green heart (*Warburgia ugandensis* Sprague) was weighed, crushed in mortar and then transferred into 250 ml Pyrex conical flasks containing 150 ml of de-ionised water. The leaves were infused by boiling at 100° C for 20 minutes and then filtered through a muslin cloth. Then seven and half grams of malt extract agar was added to the infusion and autoclaved at 121° C for 15 minutes. Twenty ml of warm MEA-infusion mixture was poured into each 9 cm diameter sterile plastic disposable petri plates. For biomass accumulation assessment, 60 g of fresh crushed leaves of each plant was transferred to 1000 ml conical flask containing 600 ml de-ionised water. Leaves were infused as described before for solid medium evaluation. Fifteen grams of malt extract were added to the infusion. One hundred and fifty ml infusion-malt extract from each plant was dispensed into three, 250 ml Pyrex conical flask to make three replicates and sterilised under pressure at 121° C for 15 minutes. MEA-infusion mixture and ME-infusion were both adjusted to pH 6.5 prior to sterilisation. The liquid ME and MEA plates without any plant extract served as control. Inoculation, harvesting of biomass and other observations were made as described earlier.

RESULTS AND DISCUSSION

Phomopsis theae is a pycnidial pathogen and produces pycnidia both in culture and on cankered part of the tea (Otieno, 1998). In the present study also *P. theae* produced pycnidia which were black, of 0.5-1mm diameter, and densely populated towards the center of the mycelial growth on MEA plates. Good mycelial growth produced maximum number of pycnidia whereas the rest less mycelial growth was observed with fewer pycnidia.

Results on the effect of copper oxychoride, mancozeb and benomyl on the mycelial growth of and pycnidial production by *P. theae* are presented in Table 1. There were significant differences ($P \leq 0.01$) except in few cases, between the three fungicides and their concentrations on the growth of two isolates, P228 and P794, of the fungus in both malt-extract agar and liquid malt-extract. Maximum radial growth (with abundant pycnidial production) equal to or even higher than in the control occurred in medium amended with copper oxychoride at 10 ppm and 25 ppm for P228, and at all concentrations for P794. There were no significant differences ($P \geq 0.01$) between the radial mycelial growth at 25 ppm and 50 ppm for P794. The percentage increase of growth over control was 15.5 at 25 ppm for P228 and 8.1, 9.8, 20.7 for P794 at 25, 50 and 100 ppm, respectively. The increase in dry weight of biomass over control was observed at all concentrations of copper oxycloride. However, they were not significantly different from each other. The pathogen produced highest number of pycnidia in medium amended with copper oxycloride, showing its ineffectiveness in reducing inoculum of the disease. These findings suggest that copper oxycloride was not effective as it did not cause substantial growth inhibition of the two isolates of the pathogen, instead it apparently stimulated growth in some cases. The increase in growth over control may be due to iatrogenic effects of copper on the pathogen. Such effect was also observed in coffee against *Pseudomonas syringae* (coffee bacterial pathogen) where captafol sprays encouraged the growth of the pathogen (Kairu *et al.*, 1984).

Mancozeb at 10 ppm and 25 ppm was less effective, though the radial mycelial growth and biomass accumulation were inhibited in P228, compared with the control. However, at 50 ppm and 100 ppm, the radial mycelial growth was substantially inhibited in P228 while no growth occurred in P794. These inhibitions were highly significant ($P \leq 0.01$) suggesting that mancozeb

inhibited growth only at higher concentrations under *in vitro* conditions. At 50 ppm and 100 ppm of mancozeb, P228 produced a few pycnidia while P794 did not produce even a single pycnidium. Arulpragasam (1989) found protectant fungicides ineffective against *P. theae* since they could not penetrate the pathogen growing inside the bark.

Benomyl at all its concentrations completely inhibited the growth, the pycnidial production and biomass accumulation of *P. theae,* achieving 100% inhibition over control. Shanmuganathan and Bopearatchy (1972) also reported that benomyl at 20 ppm completely inhibited conidial germination of *P. theae.*

The results presented in Table 2 show that 10% aqueous leaf extracts of four indigenous medicinal plants, *Lippia javanicum, Urtica massaica, Satureia biflora* and *Warburgia ugandensis* were inhibitory to the growth of the two isolates of *P. theae* at different degrees both in solid and liquid media. Compared with the control, the extracts of *W. ugandensis* affected 78% and 100% inhibition of radial mycelial growth and biomass accumulation, respectively, in P228 and 100% inhibition of both parameters in P794. The fungicidal properties of *W. ugandensis* may be due to presence of warburganal, muzigadial and polygodial which are potential antifungal substances (Kubo *et al.*, 1977; Kubo and

TABLE 1. Growth of two isolates of *P. theae,* P228 and P794 on MEA (mm) and biomass accumulation (g) on liquid ME containing different concentration of three fungicides at 20±2°C

Fungicides	Concentration	P228				P794		
		Mycelial growth (mm)		Pycnidial production	Biomass accumulation (g)	Mycelial growth (mm)	Pycnidial production	Biomass accumulation (g)
		8 days	14 days		1 days	8 days		21 days
	10 ppm	28.3a	41.3b	++++	0.77a	59.5 C	++++	0.99a
		(0.7)	(1.7)		(8.5)*	(0)		(21.4)
	25 ppm	32.5a	48.5a	+++	0.80a	64.3b	+++	0.77a
Copper oxychloride	50 ppm	23.3b	34.5c	++	0.76a	65.3b	++++	0.84a
		(18.2)	(17.9)		(7.1)*	(9.8)*		(33.3)
	100 ppm	28.5a	36.0b	+	0.80a	71.8a	++++	1.08b
		(0)	(14.3)		(12.7)*	(20.7)*		(14.3)
	10 ppm	22.3b	33.3c	+++	0.76a	45.0d	++	0.80b
		(21.8)	(20.7)		(7.1)*	(24.4)		(36.5)
	25 ppm	17.3c	26.5d	++	0.80a	45.0d	+++	0.67a
Mancozeb		(39.3)	(36.9)		(12.7)*	(24.4)		(46.8)
	50 ppm	15.5c	22.8d	+	0.57b	0e	-	0.60b
		(45.6)	(45.7)		(19.7)	(100)		(52.4)
	100 ppm	4.0d	10.3e	+	0.36b	0e	-	0c
		(85.9)	(75.5)		(49.3)	(100)		(100)
	10 ppm	0d	0f	-	0c	0e	-	0c
		(100)	(100)		(100)	(100)		(100)
	25 ppm	0d	0f	-	0c	0e	-	0c
Benomyl		(100)	(100)		(100)	(100)		(100)
	50 ppm	0d	0f	-	0c	0e	-	0c
		(100)	(100)		(100)	(100)		(100)
	100 ppm	0d	0f	-	0c	0e	-	0c
		(100)	(100)		(100)	(100)		(100)
Control		28.5a	42b	++++	0.71a	59.5c	++++	1.26a
CV %		13.94	12.75		22.48	6.59		31.42

In a column means followed by the same letter are not significantly different from each other at P≥ 0.01 level
Figures in parenthesis are inhibition percentage and in parenthesis with asterisk are percentage increase over control
Pycnidial production: Abundant ++++ (above 50); good +++ (30-50); moderate ++ (15-30); scanty + (below 15); nil

TABLE 2. Growth of two isolates of *P. theae*, P228 and P794 on MEA (mm) and biomass accumulation (g) in liquid ME containing aqueous leaf extracts from different indigenous plants at 20± 2°C

Plants (10% leaf extracts)	P228				P794		
	Mycelial growth (mm)		Pycnidial production	Biomass accumulation (g)	Mycelial growth (mm)	Pycnidial production	Biomass accumulation (g)
	8 days	14 days		21 days	8 days		
Lippea javanica	19.0 (39.3)	33.0c (33.3)	++++	0.22b (48.8)	48.5b (38.2)	+++	0.25b (74.8)
Urtica massaica	25.3b (19.2)	38.0b (23.2)		0.23b (46.5)	37.5c (52.2)	++	0.17b (82.8)
Saturea biflora	32.5a (3.81)	44.8a (9.5)	++	0.35a (18.6)	53.5d (31.9)	++++	0.29b (70.7)
Warburgia ugandensis	5.3d (83.1)	11.0d (77.8)	+	0c (100)	0c (100)	-	0c (100)
Control	31.3a	49.5a	++++	0.43a	78.5a	++++	0.99a
C.V %	16.44	18.32		23.39	19.36		10.93

In a column means followed by the same letter are not significantly different from each other at P≤ 0.05 level.
Figures in parenthesis are inhibition percentage over control.
Pycnidial production: Abundant ++++ (above 50); good +++ (30-50); moderate ++ (15-30); scanty + (below 15); nil

Taniguchi, 1988). Exacts of *U. massaica* and *L. javanicum* were less effective as compared to extracts of *W. ugandensis,* with an average of 50% inhibition over control. Aqeous leaf extracts of *S. biflora* was the least effective as compared to others in inhibiting the growth of the two isolates of *P. theae*. Although this study illuminates the effect of various fungicides, it reminas unclear whether fungicide concentrations outside the considered ranges would exhibit different results. Further work is needed to study the effect of higher concentrations of these extracts especially those that exhibited limited exhibitory effects. The effect of different concentrations of *W. ugandensis* on the growth showed that 3.33% (20 g/600 ml of water) of aqueous leaf extracts was minimum to get 100% inhibition over control for both isolates of *P. theae.* These results suggest that leaf extracts of *W. ugandensis* are as effective as benomyl in inhibiting the growth of the pathogen.

The use of synthetic fungicides on consumable plants and plant parts, such as tea leaves, is generally discouraged because of its potential risk on human health and the environment. During recent years research works have been geared towards the use of plant-derived substances for the control of plant pathogens. It has been found that certain plant extracts have fungitoxic properties, and few of them have been tested with success under glasshouse and field conditions (Reimers *et al.*, 1993; Singh *et al.*, 1995; Singh *et al.*, 2000; Varshney, 2001). In the present study, 10% aqueous leaf extracts of *W. ugandensis* compared favourably with benomyl under *in vitro* conditions and this is aplausable aspect for futher evaluation to explore its efficacy on the disease under screen house and under field conditions.

ACKNOWLEDGEMENT

This study was conducted at the Tea Research Foundation of Kenya, Kericho and we are grateful for the support and access to all facilities needed to conduct this work.

REFERENCES

Anon. 2001. Annual Bulletin Statistics, International tea committee, 30-32.

Arulpragasam, P.V. 1989. Studies on the low country stem diseases of tea: In science and practice in tea culture. *Tea Research Foundation,* Calcutta. pp. 120-130.

De Lima, C.P.F., Ondieki, J.J., Mbogo, O.J. and Okioma, S.W. 1978. A summary report on a survey of tea pests and diseases in Kenya. *Tea in East Africa* 18:20-25.

Fordham, R. 1971. Stomatal physiology and water relations of the tea bush. In: water and the tea plants (Eds. Carr, M.K.V. and Carr, S.). *Tea Research Institute of East Africa.* pp. 89-100.

Haster, D.N. 1973. Notes on collar and branch canker of tea (*Phomopsis theae*) in Mfindi Tanzania, *Tea in East Africa* 2:9-12.

Kairu, G.M., Nyangena, C.M.S., Javed, Z.U.R . and Crose, J.E. 1984. Iatrogenic effects of captafol on bacterial blight of coffee. *Plant Pathology* 33:131-132.

Kubo, I., Miura, I., Pettei, M.J. and Lee, Y.W. 1977. Muzigadial and Warburganal, potent antifungal, antiyeast and African army worm antifeedant agents. *Tetrahedron Letters* No. 52:4553-4556.

Kubo, I. and Taniguchi, M. 1988. Polygodial, an antifungal potentiator. *Journal of Natural Products* 51:22-29.

Oniong'o, M.O. 2003. Effects of some fungicides, plant extracts of indigenous plants and host genotypes on *in vitro* growth of *Phomopsis theae* Petch, the cause of stem canker disease of tea. MSc. thesis, Egerton University. pp. 79.

Onsando, J.M. 1988. Tea diseases situation in Kisii district. *Tea* 9:47-49.

Otieno, W. 1998. Stem canker disease of tea-causal organism *Phomopsis theae* Petch *TRFK Quarterly Bulletin* 3:4-7.

Otieno, W. 2001. Plant pathological research for sustainable tea production achievements prospects and limitation. *Tea Research of Kenya.* pp. 41.

Reimers, F., Smolka, S.E., Werres, S., Schumacher, K.P. and Wagner, K.G. 1993. Effect of ajoene, a compound derived from *Allium sativum,* on phytopathogenic and epiphytic microorganisms, *Z. Pflkrankh und Pflschutz* 100: 622-633.

Shanmuganathan, N. and Bopearatchy, R.N. 1972. Uptake of benomyl by tea plants and its effectiveness against stem canker caused by *Phomopsis theae* Petch. *Tea Quarterly Bulletin* 43:103-110.

Shanmuganathan, N. and De Silva, R. 1968. Susceptibility of tea clones to collar and branch canker disease of tea. *Tea Quarterly Bulletin* 39:92-97.

Shanmuganathan, N. and Rodrigo, W.R.F. 1966. Studies on collar and branch canker of young tea caused by *Phomopsis theae* Petch II. Influence of soil moisture to the disease. *Tea quarterly* 38:320-330.

Singh, U.P., Prithiviraj, B., Wagner, K.P. and Schumacher 1995. Effect of ajoene, a constituent of garlic (*Allium sativum*), on powdery mildew (*Erysiphe pisi*) of Pea (*Pisum sativum*). *Z. Pflkrankh. und Pflschutz* 102: 399-406.

Singh, U.P., Prithiviraj, B., Singh, K.P. and Sharma, B.K. 2000. Control of powdery mildew (*Erysiphe pisi*) of pea (*Pisum sativum*) by combined application of plant growth-promoting rhizobacteria and Neemazal. *Z. Pflkrankh. und Pflschutz.* 107:59-66.

Steele, R.D.G., Torrie, J.H. and Dickey, D.A. 1997. Principles and Procedures of Statistics: A Biometrical Approach. 3rd ed. McGraw-hill, New York. 665pp.

Varshney, V. 2001. Effect of plant extracts on *Dreschslera graminae,* the causal agent of stripe disease of barley. *Indian Phytopathology* 54:88-90.

Willat, S.T. 1970. A comparative study on the development of young tea under irrigation in the field. *Tropical Agriculture* 47:243-249.