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The efficacy of sterilizing agents, copper oxychloride, vegetable oil and agrowipe (botanic neem extract) against crown gall disease of roses in Kericho, Kenya

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Abstract

The experiments were conducted at James Finlay Kenya, flowers division Tarakwet farm in Kericho county from January 2016 to December 2016 to study the efficacy of copper oxychloride 1.0 g/L, hydrogen peroxide 1.0 mL/L, dettol 0.5 mL/L and 1.0 mL/L, agrowipe undiluted and fresh fri undiluted as control agents of crown gall disease in roses. Crown gall causes losses of between 75-95% on susceptible rose varieties hence the need to develop effective control strategies. Results showed plots treated with agrowipe and fresh fri had higher yield of roses, which were of better quality, longer, heavier and also inhibited growth of crown gall tumors and fresh crown gall growths compared to copper oxychloride at 1.0 g/L and untreated control. Dettol at 0.5 mL/L, 1.0 mL/L and hydrogen peroxide 1.0 mL/L had moderate yield of roses and crown gall control. Similar results were observed in the pot trials. Agrowipe and fresh fri effectively controlled crown gall diseases hence were recommended for use in controlling crown gall disease in roses in Kenya.

Keywords: *Agrowipe, Crown gall, Control agents, Tumor, Roses*

1. Introduction

Physical agents such as heat and ultraviolent light, chemical agents such as disinfectants and antibiotics have been used to prevent bacterial contamination and spread (Hauser, 2013). Disinfectants are chemical substances that kill or retard growth of microorganisms while antibiotics are substances produced by living microbes that inhibit growth of microbes (McManus and Stockwell, 2001). Bacteria strains resistant to copper compounds are common, in addition copper compounds can also be phytotoxic on certain plant species (Alsup, 2004). Natural products also known as botanical pesticides which exhibit antimicrobial activity have been used for a long time in controlling microorganisms causing plant and human diseases (Mitali *et al*., 2012). Kenya is now a lead exporter of cut flowers to the European Union with a market share of about 38% (Kenya Flower Council, 2019). In 2018, the export of flowers from Kenya earned the country Ksh.113.16 billion up from Ksh.

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82.25 billion in 2017, representing 38% growth on export volume (Kenya Flower Council, 2019). In Africa, Kenya is one of the most prominent fresh flower exporting countries. Floriculture is the most developed sector and accounts for about 74% of all horticultural export (Kenya Flower Council, 2019). Despite the tremendous contribution to the Kenyan economy, profitable production of roses is constrained by a number of plant diseases such as crown gall caused by *Agrobacterium tumefasciens* (Horst, 1983). Rose plants infected by *A. tumefasciens* manifest slower growth, stunting, yellowing, chlorotic leaves and fail to produce healthy flowers. The severely infected plants develop sensitivity to environmental stress (Kado, 2002). The pathogenicity of *A. tumefasciens* is caused by the Tumor Inducing (Ti) plasmid, located in its cytoplasm. The pathogen can survive in the soil for more than 10 years. Severe infection kills the plant (Collins, 2001). Roses that have crown gall develop rounded tumors which are brown to brownish black in color and they develop from smooth spongy to rough texture with age. Expanding tumors destroy adjacent healthy tissue and prevent normal flow of water and nutrients (Kado, 2002).

A survey done in East Africa in 2013 on the losses caused by *A. tumefasciens* showed that crown gall incidence on susceptible varieties was between 75-95% when the crop was only three years old (Maina *et al.*, 2013). A number of measures have been adopted to reduce the impact of *A. tumefasciens* in production of roses. However, no method has been effective in reducing crown gall completely. Current rose management regimes in Kenya lack protocol for this disease hence farmers resort to untested solutions (Maina *et al*., 2013). In addition, there are no registered pesticides in Kenya and in East Africa for controlling the disease. Control measures used by rose growers to minimize the effects of *A. tumefasciens* in roses include; observing field hygiene, regular disinfection of pruning and harvesting tools such as secateurs, controlling root chewing insects and piercing nematodes, rouging of infected plants (Kado, 2002); biocontrol with the non-pathogenic organism *Agrobacterium radiobacter* strain K 84 (Maarten *et al.*, 1987). However, rose plants treated with dygall (*A. radiobacter*) twice in one flower farm in Kenya developed crown gall tumors (Maina *et al.*, 2013). There are no recent studies reported on control methods of *A. tumefasciens*. The present paper reports on the study on the efficacy of copper oxychloride, sterilizing agents (hydrogen peroxide 50% and dettol-chloroxylenol 4.8%), vegetable oil and a local botanic neem extract (Agrowipe) found in Kenya as control agents of crown gall disease in roses. These results provide suitable management strategies for the control of *A. tumefasciens* that can be used in the floriculture industry both in Kenya and East Africa.

2. Materials and methods

2.1. Green house trials

The experiment was conducted at Finlay Flowers Kenya, Tarakwet farm in Kericho county, located at 0 ° 27 south / 35° 27 east and 2,100 meters above sea level. Average maximum and minimum temperatures range from 19-22°C, respectively with a total annual rainfall ranging from 2,000 to 2,500 mm. The experiment was conducted from January 2016 to December 2016 in a commercial greenhouse (F36) planted with four years old rose variety *Tropical amazone* that is susceptible to crown gall infection. The rose plants were already infected with crown gall disease. The roses were grown hydroponically on pumice media at a pH range of 5.8-6.2.

2.2. Experimental design and treatment method

A method described by Biondi *et al*. (2009) was used. The experiment was arranged in a Randomized Complete Block Design (RCBD) replicated four times, giving a total of 28 plots. Each plot comprised 40 rose plants measuring 2 × 1 m^2 with guard rows of 1 m^2 between plots. Ten galls were tagged on each treatment replicate and the initial diameter of the galls measured using a Vanier calipers. The galls were then cut using a sterilized roll cut (roll cut was sterilized by dipping into spore kill solution (Didocyldimethyl ammonium chloride) before cutting each gall). Seven treatments consisting of Isacop 50 WP (copper oxychloride 1.0 g / L, fresh fry (vegetable oil) undiluted, agrowipe (botanic neem extract) undiluted, dettol (chloroxylenol 4.8%) 0.5 mL / L and 1.0 mL / L, hydrogen peroxide 50% 1.0 mL / L and control (water only) were applied on wounds once a month. The vegetable oil and botanic neem extract were applied using a paint brush while hydrogen peroxide 1.0 mL / L, chloroxylenol 4.8% 0.5 and 1.0 mL / L, copper oxychloride and water were applied using a hand sprayer 1.5 L (Hardi Ltd, Kenya).Treatments were applied for 12 months.

2.3. Pot experiment

In order to monitor the growth of crown gall tumors closely, a pot trial was conducted. A method described by Artur *et al*. (2012) was used for the experiment. Rose seedlings grafted on natal briar root stock variety *Tropical*

amazone already infected with crown gall were used for the experiment. The seedlings were grown in plastic containers measuring (30 cm ×15 cm × 30 cm) filled with pumice. The initial diameter of the galls was measured using a Vanier caliper. The galls were cut as described in the field experiment and similar treatments applied at the rates shown. Treatments comprised of three plants replicated four times with replicates arranged in a Completely Randomized Design (CRD).

2.4. Data collection

Fresh crown gall growths were counted and removed once a month for a period of 12 months. Tumor diameter of the tagged galls was also measured once a month for a period of 12 months. Mean tumor weight of the fresh galls was determined after 12 months by cutting and weighing them using servo weighing scale model SB 3000 (Servo Balans Ltd). Yield was determined by counting total number of marketable stems from each treatment replicate on a daily basis for a period of 12 months. The quality of stems produced was determined by sampling 30 stems once a week from each treatment replicate and the following parameters measured; stem length (using ruler), stem weight (using weighing balance), flower head size (was measured using a Vanier calipers). Other pests and diseases were controlled when encountered and other agronomic operations such as weeding, removal of blind shoots and bloomers were carried throughout the experimental period. Fertilizers comprising both macro and micro elements were administered on a daily basis through the fertigation station as per the feeding program (Table 1). Macro elements were mixed in tank A while micro elements were mixed in tank B except iron since it binds with the sulphates. Both tanks had a capacity of 500 L. The final solution had an E.C of 1.9 and a pH range of 5.5-6.2.

Table 1: Roses feeding program used at Finlay flowers Kericho

Type of Fertilizer	**Quantity (Kilograms)**
Tank A (macro elements)	
Calcium nitrate	72
Magnesium nitrate	10
Potassium nitrate	10
Micrel iron	1.6
Tank B (micro elements)	
Potassium nitrate	18
Magnesium nitrate	25
Mono potassium phosphate	11
Potassium nitrate	10
Mono ammonium phosphate	8
Copper 140 EDTA chelate	60
Sodium molybdate	395
Manganese 130 EDTA chelate	300
Zinc sulphate	100
Potassium borate	150

2.5. Statistical data analyses

Data was subjected to statistical analysis using the GENSTAT software.

3. Results

Results on the effect of copper oxychloride, vegetable oil, botanic neem extract, chloroxylenol 4.8% and hydrogen peroxide on new galls, tumor weight, tumor diameter are shown in Tables 2-5. There were significant differences ($p \leq 0.05$) on the yield and quality of marketable rose stems on plots treated with vegetable oil (undiluted), botanic neem extract (undiluted) and copper oxychloride at 1.0 g/L. Plots treated with the botanic neem extract and the vegetable oil had a higher yield of marketable roses with better quality (that is they were longer and heavier) compared to plots treated with copper oxychloride 1.0 g/L and untreated control. Plots treated with chloroxylenol 4.8% 0.5 mL/L, 1.0 mL/L and hydrogen peroxide had moderate yield of marketable stems. Results also show that undiluted vegetable oil and botanic neem extract effectively inhibited growth of crown gall tumors and fresh galls compared to copper oxychloride 1.0 g/L and untreated control plots at ($p \leq 0.05$). These were observed both in the field and pot trials.

Table 2: Efficacy of Isacop 50 WP (copper oxychloride), fresh fri (vegetable oil), agrowipe (botanic neem extract), dettol (chloroxylenol 4.8%), hydrogen peroxide 50% and untreated control on yield of roses variety *tropical amazone* (pooled data for one year)

Treatment	Yield of marketable stems
Isacop 50 WP (copper oxychloride) 1.0 g/L	446.0[b]
Fresh fri (vegetable oil) – undiluted	510.8[a]
Agrowipe (botanic neem extract – undiluted	508[a]
Dettol (chloroxylenol 4.8%) 0.5 mL/ L	491.3[ab]
Dettol (chloroxylenol 4.8%) 1.0 mL/ L	471.5[ab]
Hydrogen peroxide 50% 1.0 mL/ L	500.5[ab]
Untreated control	495.0[ab]

Note: [b]: significant difference in means compared [a, ab] no significant differences in means at $p < 0.05$, a: significant difference compared to the other treatments. One way ANOVA followed by Turkey multiple comparison test.

Table 3: Efficacy of Isacop 50 WP (copper oxychloride), fresh fri (vegetable oil), agrowipe (botanic neem extract), dettol (chloroxylenol 4.8%), hydrogen peroxide 50% and untreated control on crown gall tumors rose variety *tropical amazone* (pooled data for one year)

Treatment	Mean tumor diameter (CM)	Mean tumor weight (G)	Mean new galls
Isacop 50 WP (copper oxychloride) 1.0 g/L	10.0[ab]	36.0[a]	415.3[a]
Fresh fri (vegetable oil) – undiluted	0.5[e]	1.5[b]	272.2[b]
Agrowipe (botanic neem extract –undiluted	0.2[e]	0.0[b]	240.0[b]
Dettol (chloroxylenol 4.8%) 0.5 mL/L	7.8[de]	16.5[ab]	360.8[ab]
Dettol (chloroxylenol 4.8%) 1.0 mL/L	9.1[bc]	4.3[b]	465.0[ab]
Hydrogen peroxide 50% 1.0 mL/L	7.0[a]	2.8[b]	328.3[ab]
Untreated control	11.7[a]	10.0[ab]	434.0[a]

Note: [a, b, c, d, e] means significantly different from each other, ab, de, bc, not significant differences in means at $p < 0.05$.

Table 4: Efficacy of Isacop 50 WP (copper oxychloride), fresh fri (vegetable oil), agrowipe (botanic neem extract), dettol (chloroxylenol 4.8%), hydrogen peroxide 50% and untreated control on quality of roses variety *tropical amazone* (pooled data for one year)

Treatment	Mean stem length (cm)	Mean head size (cm)	Mean stem weight (g)
Isacop 50 WP (copper oxychloride) 1.0 g/L	54.0[a]	3.4[b]	23.0[a]
Fresh fri (vegetable oil) – undiluted	59.0[b]	4.1[ab]	28.5[b]
Agrowipe (botanic neem extract – undiluted	59.0[b]	4.1[ab]	28.3[b]
Dettol (chloroxylenol 4.8%) 0.5 mL/L	59.0[b]	4.3[a]	27.3[b]
Dettol (chloroxylenol 4.8%) 1.0 mL/L	57.5[b]	4.3[a]	8.3[b]
Hydrogen peroxide 50% 1.0 mL/L	58.8[b]	4.3[a]	28.5[b]
Untreated control	55.2[a]	3.3[b]	23.3[a]

Note: [a]: means significantly different from [b, ab] means not significantly different from each other $p < 0.05$. One way ANOVA followed by Turkeys multiple comparison test.

Table 5: Efficacy of Isacop 50 WP (copper oxychloride), fresh fri (vegetable oil), agrowipe (botanic neem extract), dettol (chloroxylenol 4.8%), hydrogen peroxide 50% and untreated control on crown gall tumors rose variety *tropical amazone* (pooled data for one year pot experiment)

Treatment	Mean tumor diameter (cm)	Mean tumor weight (cm)
Isacop 50 WP (copper oxychloride) 1.0 g/L	14.7[a]	36.0[a]
Fresh fri (vegetable oil) – undiluted	3.1[b]	1.5[b]
Agrowipe (botanic neem extract – undiluted	0.9[b]	0.0[b]
Dettol (chloroxylenol 4.8%) 0.5 mL/L	8.1[ab]	16.5[ab]
Dettol (chloroxylenol 4.8%) 1.0 mL/L	8.9[ab]	14.3[ab]
Hydrogen peroxide 50% 1.0 mL/L	6.2[ab]	12.8[ab]
Untreated control	8.3[ab]	10.0[ab]

Note: [a]: means significantly different from [b, ab] means not significantly different from each other at $p < 0.05$ one way ANOVA followed by Turkeys multiple comparison test.

4. Discussion

Vegetable oil and botanic neem extract (Agrowipe) showed antibacterial activity against *A. tumefasciens.* Agrowipe is a biological and herbal solution made from African herbal trees, seeds shrubs and weeds mixed together. The efficacy of different plant extracts belonging to different plant species against fungal and bacterial diseases have been reported under laboratory conditions (Oniang'o *et al.*, 2005 and Mitali *et al.*, 2012). The inhibitory effect of the botanic neem extract might be due to presence of secondary metabolites as reported by Mitali *et al.* (2012), while the vegetable oil is thought to suffocate the crown gall as was shown by Maina *et al.* (2013). However, further work is needed to establish the exact mode of action of the vegetable oil and botanic neem extract in controlling *A. tumefasciens.* Contrary to this, crown gall tumors increased in size and weight and more new galls grew on plots treated with copper oxychloride at 1.0 g/L, showing its ineffectiveness in controlling *A. tumefasciens.* These findings suggest that copper oxychloride was not effective as it did not suppress growth of crown gall tumors, but instead, it apparently stimulated growth. The increase in crown gall growth over control may be due to iatrogenic effects of copper on the pathogen. Such effect was also

observed in coffee against *Pseudomonas syringae* where captafol sprays encouraged growth of the pathogen (Kairu *et al.*, 1984) and in tea against *Phomopsis thea* where copper oxychloride encouraged growth of the pathogen *in vitro* (Oniang'o *et al*., 2005). Hydrogen peroxide at 1.0 and chloroxylenol 4.8%) at 0.5 mL / L and 1.0% moderately inhibited growth of crown gall tumors and had a better quality of marketable rose stems compared to copper oxychloride at 1.0 g / L. The significant differences were observed at ($p \geq 0.05$).

Because of high costs of chemical pesticides, and their hazardous consequences, the use of different biodegradable materials like fresh plant extracts has gained importance during the last three decades (Reimers *et al*., 1993; Singh *et al*., 1995; Singh *et al* 1999; Varshney, 2001 and Oniang'o *et al.*, 2005). In addition, the use of antibiotics in controlling bacterial plant diseases is highly discouraged because they can develop resistance (McManus and Stockwell, 2001).

5. Conclusion

The findings of this study suggest that botanical neem extract (undiluted) and vegetable oil (undiluted) effectively controlled *A. tumefasciens* and should be adopted for use in controlling crown gall disease in Roses.

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References

Alsup, C. M. (2004). Screening for active ingredients in plant extracts that inhibit growth of *Agrobacterium tumefasciens*. *The Plant Health Instructor.* doi:10.1094 / PHL-'1-2004-0226-01

Artur, M., Piotr, S. and Stanisaw, B. (2012). Efficacy of fungicides and essential oils against bacterial diseases in fruit trees. *Journal of Plant Protection Research.* 52(4), 55-56.

Biondi, E., Bini, F., Anaclerio, F. and Bazzi, C. (2009). Effect of bio agents and resistance inducers on grapevine crown gall. *Phytopathol. Mediterr*. 48, 379-384.

Collins, A. (2001). A Class Project for PP 728 Soil - Borne Plant Pathogens. North Carolina State University Department of Plant Pathology.

Hauser, J. T. (2013). Techniques for studying bacteria and fungi. *Carolina Biological supply Company*. Burlinton, N.C.

Horst, R. K. (1983). Compendium of Rose Diseases. Academic Press, St. Paul, MN. pp. 23-25.

Kado, C. I. (2002). Crown gall tumors. In S. Brenner and J. H. Miller (Eds.) *Encyclopedia of Genetics*. Academic Press, San Diego, CA 1-3.

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.

Kenya Flower Council (2019). Floriculture export in Kenya. July 2019 (Web exclusive story). Retrieved from http: / / www.farmbizafrica.com.

Maarten, H., Ryder, M., John, E. S., Anna, S. and Stephen, K. F. (1987). "Genetic analysis of agrocin 84 production and immunity in *Agrobacterium* spp. *Journal of Bacteriology.* 169, 4184-4189.

Maina, G., Mutitu, E. W. and Ngaruiya, P. N. (2013). Study to review the impact on losses of competitiveness due to infestation of *Agrobacterium tumefasciens* and other soil borne diseases in Kenya, Uganda, Ethiopia and Tanzania. Retrieved from http / / www.Kenyaflowercouncil.0rg / presentation2013

McManus, P. S. and Stockwell,V. O. (2001). Antibiotic use for plant disease management in the United States. Online plant health progress. Retrieved from http: / /10.1094 / PHP-2001- 0327-01-RV

Mitali, M. P., Manoranjan, K. and Sahu, R. K. (2012). Bio efficacy of some plant extracts on growth parameters and control of diseases in *Lycopersicum esculentum*. *Asian Journal of Plant Science and Research.* 2(2), 129-142.

Oniang'o, M. O., Khare, B. K., Achwanya, O. S. and Otieno, W. (2005). Efficacy of synthetic and non synthetic fungicides against stem canker disease of tea. *African Crop Science Journal.* 13(3), 209 -214.

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. pfkrankh and pfschutz* 100, 622-633.

Singh, U. P., Prithiviraj, B., Wagner, K. B. and Plank-Shumecher, K. G. (1995). Effect of ajeone a constituent of garlic (*Allium sativum),* powdery mildew (*Erysiphe pisi*) of *pea (Pisum sativum). J. Plant. Dis. Protect.*102, 399-406.

Singh, R. S., Sharma, B. K., Srivastava, J. S., Singh, U. P. and Ray, A. B. (1999). Antifungal activity of Alstovenine, a plant alkaloid isolated from *Alstoniavenanata*. *Foliar Microbiol*. 44(5), 510-512.

Varshney, V. (2001). Effect of plant extracts on *Dreschslera graminae*, the causal agent of strip disease of barley. *Indian Phytopathology.* 54(1), 88-90.

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