# Agrigold™, an alternative disinfectant with superior bactericidal efficacy

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**Abstract:** Disinfectants are used in fruit pack house facilities to ensure good pack house hygiene and prevent postharvest disease development. Agrigold™, a copper compound, was tested as a bacterial and fungicidal disinfectant in pack houses on several subtropical crops and potatoes in South Africa. The product showed superior bactericidal efficacy and, when used in combination with a fungicide, could control postharvest fungi to extend shelf life and maintain fruit quality. Additionally, it enhanced the efficacy of fungicides that contained the active ingredients imazalil sulphate and prochloraz. Results showed that Agrigold's efficacy as a disinfectant was not dependent on pH, compatible with two fungicides commonly used for postharvest microorganism control and had a low environmental impact. The latter product could therefore be added to the range of disinfectants available to optimise the control of postharvest diseases of potato and various fruits.

**Keywords:** Agrigold; copper compound; disinfectant; postharvest; Erwinia rot; anthracnose; green mould; subtropical crops; fruit; potatoes.

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**Biographical notes:** Mieke Daneel is a senior researcher at the ARC-Institute for Tropical and Subtropical Crops (ARC-ITSC), Nelspruit South Africa. She has been doing research in the mango and other subtropical fruit pack houses for several years as the producers seem in need of better guidelines and methods for post-harvest practices. The research is funded by the farmers and is aimed at getting practical solutions for enhancing efficacy of pack house procedures. One such solution is the use of a good disinfectant in the packhouse.

## 1 Introduction

Pack house hygiene is very important in the prevention of postharvest disease development in avocado (*Persea americana* Mill.), citrus (*Citrus* L.), mango (*Mangifera indica* L.), papaya (*Carica papaya* L.) and potato (*Solanum tubersosum* L.) (Di Martino Aleppo and Lanza, 1996; Sholberg, 2004; Sholberg and Conway, 2004; Sommer, 1982). On the pack line, critical points have been identified where disinfectants

and fungicides can be added to reduce disease development, enhance fruit quality and extend shelf life (Roux and Boshoff, 1999).

When the produce arrives in the pack house, contaminants such as soil and organic debris as well as microorganisms are often found on the produce. During washing of the produce in the first bath, contaminants and microorganisms are deposited in the water. To prevent the contaminants and microorganisms to spread further on the pack line the water needs to be properly sanitised. A disinfectant will sanitise the wash water and maintain a low microbiological count in the water, ensuring that the water does not become a reservoir for bacteria (Zagory, 2000). Unfortunately disinfectants have no penetration action and cannot cure produce that has already been infected with diseases in the field whereas fungicides added to the process have a different action/function. Many of the registered postharvest fungicides can penetrate the fruit and therefore prevent disease development like anthracnose [*Colletotrichum gloeosporoides* (Penz) Penz and Sacc.] on avocado, mango and papaya, green mould (*Penicillium* Link spp.) on citrus and *Erwinia* rot [*Pectobacterium carotovorum* (Jones) Waldee (syn *Erwinia carotovora*)] on potato. Because disinfectants have no residual effect and cannot prevent recontamination with disease organisms after washing, careful handling and proper pack house sanitation programs are extremely important (Zagory, 2000).

Several disinfectants are available on the South African market including hypochlorite, chlorine dioxide, chlorine bromide, peroxyacetic acid, ozone and quaternary ammonium compounds (QACs) (Esterhuyse et al., 2000). The advantages and disadvantages of using such products depend on factors such as cost, pH dependency, corrosiveness, easiness to determine concentrations in the solution, biodegradability and their carbon footprints (Sholberg, 2004; Sholberg and Conway, 2004).

The aim of the study was therefore to determine the efficacy of Agrigold (AG) as a disinfectant to optimise the prevention of disease development in the subtropical fruit and potato industries as a postharvest application in pack houses/stores.

## 2 Materials and methods

AG is a copper compound (a.i., 52.87 g/ℓ) that is available as copper nitrate in a soluble liquid (SL) formulation with a pH of 1 in the concentrated form.

### *2.1 Pack house tests*

Tests were conducted in pack houses over three seasons with fruits such as avocado, citrus, mango and papaya as well as with a vegetable, namely potato to determine the most effective concentration of AG for disinfection and disease control. Trials consisted of testing the product in the dumping bath, which is the first bath into which fruit and vegetable tubers are submerged in pack houses. The main aim of the dumping bath is for sanitising and cleaning the fruit and vegetables. In combination with the latter bath, a fungicide bath which often is a separate bath and is situated further down the pack line is used to optimise disease suppression. Dosages of AG tested were 100, 200 and 400 ppm with or without a fungicide. In each test, untreated produce as well as fruit and potato tubers treated according to the current standard treatment that represents a QAC at 100 mℓ/100 ℓ water were included. Produce was dipped for three minutes, representing

the normal contact time, in the dumping bath in the sanitising solution while contact time for the produce in the combined fungicide and AG solution was 30 s. Afterwards, the produce was returned to the pack line to undergo the normal pack line procedure, namely waxing (for most of the subtropical fruit) and drying. At the end of the pack line, produce was sorted, packed and evaluated for disease development, fruit and potato tuber quality and shelf life.

### *2.2 Laboratory tests*

Petri-dish laboratory tests were conducted to determine the effect of AG on development of the bacterial species *P. carotovora*) as well as on the fungal species and genus *C. gloeosporoides* and *Penicillium* spp., respectively, Two-hundred-and-fifty ml solutions, containing 100, 200 and 400 ppm of AG, were prepared in Erlenmeyer flasks using autoclaved tap water. The pH of the solutions was adjusted to pH 6. One ml of the bacterial suspension, from a pure culture, was then added to each flask. Serial dilutions were subsequently made from each flask 60 and 180 min after preparation of the solutions and plated on nutrient medium. The bacterial colonies were left for 24 hours at ambient temperature in natural light after which they were counted. Each dilution was plated three times.

For fungal suspensions of *Penicillium*, 1 mℓ of the spore suspension was added to 9 mℓ of a 200 ppm AG solution with and without imazalil sulphate (67 g/100 ℓ water) and this was plated three times on a potato dextrose agar (PDA) medium 10 and 120 min after preparation of the solutions. Tests were repeated three times during different times under similar conditions as prevail for the microbial assays. Populations of the fungus *C. gloeosporoides* were cultured on PDA medium and the biological method developed by Serfontein and Serfontein (2006) was used to test the efficacy of AG, prochloraz and the combination of both the products at different concentrations in terms of disease suppression.

### *2.3 Residue analysis*

Determination of copper residues on the fruit samples was done using an atomic absorption spectrometer (AAS) accredited technique.

### *2.4 Dependency of pH and water hardness*

The effect of pH on the efficacy of AG was determined by evaluating the pack house trials as well as a laboratory test where imazalil + AG-S were tested for *Penicillium* control at pH levels of 3 and 6. The effect of water hardness on fungicidal efficacy was determined by a laboratory trial using water with different hardness levels. Citrus fruit were artificially infected with *Penicillium* (using a small metal brush dipped in spore concentration of 107 to injure the fruit), dipped for 30 s in the different solutions including imazalil, with and without AG, using water with an EC of 2.22 mS and 0.574 mS respectively, and evaluated over a period of 16 days.

### *2.5 Compatibility*

Compatibility with two fungicides namely Imazalil Sulphate (imazalil – 750 g/kg SP) and Chronos 45 EC (prochloraz – 450 g/ℓ EC) was determined using a HPLC – UV (DAD) accredited technique.

## 3 Results

In subtropical crops, a combination of disinfectants and fungicides are mostly used in the pack house whereas for potatoes, only disinfectants are used to prevent produce from developing post-harvest problems.

### *3.1 Pack house trials*

For potatoes, with the major pack-house, post-harvest disease problem being *Erwinia* rot, both AG treatments provided significantly (P = 0.05) better control at all three locations than the untreated control (Table 1). The 200 and 400 ppm AG treatments also suppressed significantly (P = 0.05) more *P. carotovorum* disease three and four weeks post-harvest at Dendron than did the registered sanitiser (QAC at 100 mℓ/100 ℓ water), which is widely used in potato pack stores. The latter trend did, however, not occur for the 200 ppm AG treatment at Nkomati five and six weeks post-harvest whereas at Belfast seven and eight weeks post-harvest disease percentage was lower in the AG-S treatments but significant differences were only observed for 400 ppm AG at eight weeks. Differences in timing of evaluation are due to different inoculum levels at the different farms.

**Table 1** Percentage potatoes developing *Pectobacterium carotovorum* after postharvest treatment with AG and QAC over time at three locations in South Africa

| *Locality* | *Dendron* | | *Nkomati* | | *Belfast* | |
|---|---|---|---|---|---|---|
| *Province* | *Limpopo Province* | | *Mpumalanga Province* | | *Mpumalanga Province* | |
| *Cultivar* | *BP1* | | *Mondial* | | *Mondial* | |
| | *Week 3* | *Week 4* | *Week 5* | *Week 6* | *Week 7* | *Week 8* |
| Untreated control | 28 a | 39 a | 24 a | 29 a | 10.7 a | 30.0 a |
| AG (200 ppm) | 0 b | 0 b | 1 d | 1 d | 2.7 b | 18.6 ab |
| AG (400 ppm) | 8 b | 8 b | 4 c | 4 c | 1.3 b | 6.0 b |
| QAC (1,000 ppm) | 13 b | 24 b | 1 b | 1 b | 3.3 b | 25.3 a |
| F pr | < 0.001 | < 0.001 | < 0.001 | < 0.001 | 0.007 | 0.023 |
| LSD | 2.824 | 3.739 | 8.10 | 9.21 | 5.368 | 15.45 |

Note: Means within a column followed by the same letter are not different (P < 0.05) according to Fisher's protected least significant difference test (each location was calculated separately).

Disease development that was primarily caused by the fungus *C. gloeosporoides* (anthracnose) was significantly ($P < 0.05$) reduced for all three AG treatments combined with a fungicide compared to the untreated control for papaya, avocado and mango 10, 14 and 21 days postharvest (Table 2). Furthermore, AG + fungicide treatments provided similar results than the standard treatment, namely QAC + F in terms of disease suppression. In original trials AG without a fungicide showed a reduction in the occurrence of anthracnose in mango but not sufficiently enough and this treatment was therefore not included in further trials (data not shown).

**Table 2** Percentage fruit showing *Colletotrichum gloeosporoides* fungal development after postharvest treatment of papaya, avocado and mango at different pack houses during 2012 in South Africa

| *Fruit* | *Papaya* | *Avocado* | *Mango* | *Mango* |
|---|---|---|---|---|
| *Cultivar* | *Tainung* | *Pinkerton* | *Keitt* | *Kent* |
| *Treatments* | *10 days* | *21 days* | *10 days* | *14 days* |
| Untreated control | 46.7 a | 58.0 a | 80.0 a | 95.0 a |
| AG 100 ppm + F | 12.5 b | 12.0 b | 45.0 b | 37.5 b |
| AG 100 ppm + F | 12.5 b | 12.0 b | 47.5 b | 37.5 b |
| AG 100 ppm + F | 6.3 b | 19.5 b | 27.5 c | 45.0 b |
| QAC + F | 18.8 b | 16.0 b | 50.0 b | 45.0 b |
| F pr | < 0.001 | 0.001 | < 0.001 | < 0.001 |
| LSD | 19.05 | 18.66 | 16.21 | 25.96 |

Notes: F = fungicide prochloraz at 75 mℓ/100 ℓ water for avocado and 180 mℓ/100 ℓ water for mango and papaya; QAC = at 100 mℓ/100 ℓ water; means within a column followed by the same letter are not different ($P < 0.05$) according to Fisher's protected least significant difference test (each location was calculated separately).

Lemon fruits naturally infested with *Penicillium* showed a significant improvement in terms of *Penicillium* green mould suppression when treated with both AG + imazalil compared to those without the latter fungicide, those treated with imazalil only as well as the untreated control fruits. Treatment of fruit with the AG + imazalil fungicide yielded zero green mould development on lemons during the evaluation time on 22 November 2011 (Table 3). During the last evaluation (4 December 2011) all treatments had significantly lower percentage fruit infested with green mould, with imazalil only providing significantly lower control than the AG 400 ppm + I treatment. In terms of the percentage marketable fruits, the two AG + imazalil treatments had significantly higher levels of marketable fruits compared to the untreated control, the imazalil treatment only as well as the AG treatment only. Similarly fruit artificially infested with *Penicillium* showed a significant improvement in imazalil efficacy when AG was added to the solution (Table 4).

**Table 3** Percentage fruit infested with *Penicillium* (green mould) after postharvest treatment for the different treatments

| *Eureka Lemon* | *% Fruit with green mould* | | *% Marketable fruit* |
|---|---|---|---|
| | *22 November 2011* | *4 December 2011* | *4 December 2011* |
| Untreated control | 21.7 a | 28.3 a | 26.7 c |
| AG 200 ppm – I | 1.7 b | 3.3 bc | 48.3 b |
| AG 200 ppm + I | 0 b | 3.3 bc | 91.7 a |
| AG 400 ppm + I | 0 b | 1.7 c | 75.0 a |
| I only | 6.7 b | 8.3 b | 28.3 b |
| F pr | 0.002 | < 0.001 | < 0.001 |
| LSD | 1.758 | 1.329 | 4.013 |

Notes: I = imazalil sulphate at 67 g/100 ℓ water, AG = Agrigold; means within a column followed by the same letter are not different ($P < 0.05$) according to Fisher's protected least significant difference test

### *3.2 Laboratory tests*

For *P. carotovorum* assays, the 400 ppm AG treatments provided 100% control after both contact periods of 60 and 80 min (data not shown). During the *Penicillium* tests, similar as in the trials with artificially-infected oranges, an enhanced efficacy of imazalil sulphate was observed when AG was added while AG did not have an inhibitory effect on *Penicillium* growth on its own, implying that a synergistic effect might be present. AG was not able to reduce *C. gloeosporoides* growth on its own but AG enhanced the efficacy of prochloraz when added to the fungicide solution.

### *3.3 Residue analysis*

Copper residue analysis of the fruit was done by adding known amounts of copper standard to portions of untreated fruit samples and analysing these concurrently with the samples. Under these circumstances the limit of quantitation (LOQ) was 0.5 mg/kg for potato, 1.0 mg/kg for avocado, citrus, papaya and 2.0 mg/kg for mango.

### *3.4 The effect of pH and hardness on efficacy of AG*

AG at the recommended concentrations did not seem to be affected by pH as it was effective at any pH used ranging between 3 and 8. Similarly, water hardness did not seem to have an effect on the disinfectant at an EC of 2.22 mS (Letsitele) and 0.574 mS (Hoedspruit) with respect to disease control of citrus fruit (Table 4). The water hardness had a slight effect on imazalil efficacy as evidenced by the percentage fruit developing green mould however the addition of AG always enhanced the efficacy of imazalil irrespective of the hardness of the water and the formulation (Table 4). Similar results were seen with prochloraz in mango pack houses.

**Table 4** Percentage fruit developing green mould for the different treatments

| *Treatments* | *Day 6* | *Day 11* | *Day 16* |
|---|---|---|---|
| L – I | 2.5 d | 40.7 b | 49.4 b |
| L – I + AG | 0.0 d | 21.0 c | 33.3 bc |
| L – IS | 0.0 d | 11.1 c | 21.0 c |
| L – IS + AG | 0.0 d | 11.1 c | 14.7 c |
| L – water | 100.0 a | 100.0 a | 100.0 a |
| L – no treatment | 85.2 c | 100.0 a | 100.0 a |
| H – IS | 0.0 d | 17.3 c | 33.3 bc |
| H – IS + AG | 0.0 d | 7.4 c | 14.8 c |
| H – water | 96.3 b | 100.0 a | 100.0 a |
| H – no treatment | 100.0 a | 100.0 a | 100.0 a |
| F pr | < 0.001 | < 0.001 | < 0.001 |
| LSD | 3.203 | 17.49 | 21.52 |

Notes: Maximum number of injuries developing rot from nine fruit at nine marks per fruit was 81; L = Letsitele, Limpopo Province – high conductivity; H = Hoedspruit, Limpopo Province – low conductivity; I = imazalil EC at 100 mℓ/100 ℓ water; AG = Agrigold at 200 ppm; IS = imazalil sulphate at 67 g/100 ℓ water; no treatm = fruit not dipped in a solution.

### *3.5 Compatibility with post-harvest fungicides and environmental impact of AG*

Compatibility tests showed that AG was compatible with prochloraz and imazalil. When AG was used at 1,000 ppm and higher in combination with imazalil, precipitation was observed as soon as the pH was above 5, however this was reversible below pH 5. As a result, recommended concentrations for AG were reduced to 400 ppm or lower, resulting in no precipitation irrespective of pH.

Chemical oxygen demand (COD) counts of AG are below 150 mg/ℓ which is below the maximum oxygen demand allowed of 200 to 1,000 mg/ℓ before waste water can be returned to the environment, a pre-requisite by many governments.

## 4 Discussion and conclusions

AG proved to be an effective disinfectant to minimise bacterial and fungal population development on subtropical fruits and potatoes. Significant differences in shelf life and disease development for potatoes (Table 1), papaya, avocado, mango (Table 2) and citrus (Table 3) were observed for AG-treated produce compared to that of untreated fruit and results were similar or better than the current standard treatments (QACs) that are applied in fruit and potato pack houses. The optimum AG-concentration that proved to be effective was 200 ppm for mango, citrus, avocado and papaya whereas for potatoes it was 400 ppm. However, results showed that when AG is used in combination with a fungicide, its concentration can be reduced to 100 ppm.

Both 200 and 400 ppm provided good control in potatoes (Table 1) but because potatoes tend to be covered with soil and debris when entering the pack store, more

effective control over a longer time period will be achieved with the higher concentrations due to the high organic load in the wash drum. Shelf life was extended well beyond the range that is requested by the farmers and quality did not deteriorate.

In the papaya and avocado industries, prochloraz is added to the pack line as a standard practice for the control of fungal diseases present on both crops. When AG was added to the pack line, either in the dumping bath on its own or in combination with the fungicide in the fungicide bath less anthracnose was present in the AG treatments (Table 2). Fruit quality was often better compared with the standard procedure as the fruit looked more appetising and had a nicer colour.

When AG concentrations were high (400 ppm), lenticels in mango and avocado became more visible, thereby reducing the quality of the fruit, while a negative effect was also observed when fruit was dipped for longer than 3 minutes in the 200 and 400 ppm concentrations since lenticels became more visible. When more mature fruit was packed, it was also observed that the fruit had a tendency to ripen more quickly as a result of AG treatments, resulting in a reduced shelf life. Therefore, for ripe fruit it might be considered to use the reduced dosage of 100 ppm AG. In the mango trials, Kent fruit had a higher sugar content than Keitt fruit at harvest and, 400 ppm AG seemed to quicken ripeness and thus shortened shelf life which resulted in higher anthracnose percentage as can be seen in Table 2. However, untreated fruit always had a significantly higher percentage anthracnose.

Studies on citrus showed that fruit treated with a combination of AG and imazalil sulphate provided better control than fruit treated with imazalil sulphate on its own. Less fruit developed green mould and more fruit was marketable for a longer period (Table 3)

Studies on mango showed that the combination of AG and prochloraz seemed to enhance the efficacy of prochloraz in the control of anthracnose. It is suggested that the prochloraz was available at higher concentrations for a longer period in the fungicide bath (Daneel, 2011). Stripping problems, in which a fungicide seems to decrease in the solution more quickly than expected, have occurred in mango pack houses previously due to several reasons but of which a build-up of bacterial populations in the fungicide bath was believed to be an important factor (Swart and Broekhuizen, 2003; Swart et al., 2004). Resulting from this study and previous studies (Daneel, 2011), it would seem as if AG reduced bacterial development in the fungicide bath and thus enhanced prochloraz activity.

Residue levels as a result of dipping fruit and potato in AG treatments were always below the allowed maximum residue levels (MRL) for copper for the South African industry (MRL of 20 ppm for all crops except for potato with a MRL of 1 ppm). The latter tendency furthermore accentuates the valuable contribution that AG can make to the local fruit and potato industries.

Additionally, in situations where untreated water is used, and which is often contaminated with bacteria such as *Escherichia coli* and coliforms, the product is 100% effective in eliminating contaminants before produce has even entered the pack line.

Another benefit of AG is that the product is not pH-dependent as has been illustrated in this study. This characteristic makes it better suited than several other products that are highly dependent on pH, including many chlorine-based products (Nakagawara et al., 1998). Because the activity of AG is not dependent on pH and organic load in wash water, it can be easily tested with test strips indicating parts per million (ppm). According to such test-strip readings of which lighter colour indicates lower ppm of the product

being present, additional quantities of the product can be added to the water/fungicide bath.

Chlorine-based compounds are known for their corrosiveness (Schmidt, 2012), however, AG is classified as slightly corrosive. It is important to take environmental impact into account when evaluating a new product as this will determine the viability and sustainability of such a product. Although highly toxic to aquatic organisms in its undiluted form, AG seemed to have little long-term effect when used in diluted concentrations. In the soil, no residual effect was observed and growth enhancement was observed in tests conducted in the glass house. The product has a low COD count (Clescerl et al., 1999) and therefore lower environmental impact or lower amount of pollution when released into the system compared with other disinfectants like QACs.

This study confirmed the importance of using a sanitising agent such as AG in the pack house procedure as it results in high quality and extended shelf life of various fruits and potato compared with those that are treated by standard protocols that are currently used.

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