BIBLIOGRAPHY

MATIW, AGATE VICTRICIA P. MARCH 2013. Microbial Antagonists (MA's) and Organic-based Products as Potential Biopesticides against Black Leaf Spot (*Xanthomonas campestris pv. vitians*) of Lettuce (*Lactuca sativa*). Benguet State University, La Trinidad, Benguet.

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ABSTRACT

The study aimed to; 1. investigate the *in-vitro* activity of ten (10) microbial antagonists against *X. campestris pv. vitians*, 2. determine the effect of each microbial antagonists on the germination and seedling vigor of lettuce seedlings, 3. investigate the *in-vitro* activity of five (5) aqueous plant extracts with three (3) different extracting solvents at different concentrations against *X. campestris pv. vitians*, and 4. identify the phytochemicals present in the extracts using different extracting solvents.

Results of the *in-vitro* activity of ten bacterial antagonists against *X. campestris pv. vitians* revealed the significant inhibitory effect of PCN-2011-004 on the growth of the pathogen. This was comparable to the standard biofungicide, Virtuoso and Isolate 158. Although lower in efficacy, PCN-2011-002 and PCN-2011-005 also effected a growth suppression of *X. campestris pv. vitians*.

The highest germination was noted in Isolate 131. However, this was comparable to all the microbial antagonists tested and the standard treatments. The lowest percentage germination was obtained from the untreated seeds.

The highest mean height was noted in STR-2011-001 treated seeds. Nevertheless, this did not significantly differ from Virtuoso, Isolate 131, Isolate 94, Isolate 158, PCN-2011-003 and PCN-2011-005. On the other hand, Isolate 73, PCN-2011-002, PCN-2011-004 and Isolate 31 gave significantly shc ings than the above microbial antagonists but differed significantly from sterile distilled water treated seeds.

The main effect of plant extracts on the growth of *X. campestris pv. vitians* was not significant. Significant differences were however observed on the different extractants. Vinegar was the most effective but it did not significantly differ from wine. The least effective was water. On the other hand, the highest concentration (1:1) showed significantly the highest inhibition zone. This was comparable to 1:5 and 1:10 concentrations but was significantly different from 1:15. No significant interaction was noted among the factors evaluated.

The phytochemical analysis revealed that alkaloids, diterpenes, triterpenes and flavonoids can be extracted by water. Triterpenes, diterpenes and flavonoids can be extracted by wine. On the other hand, diterpenes, triterpenes, phenolics and flavonoids can be extracted by vinegar.

RESULTS AND DISCUSSION

Sensitivity Test of *X. campestris pv. vitians* to different microbial antagonists (MA's)

Among the microbial antagonists tested, PCN-2011-004 gave the highest inhibition zone followed by Isolate 158 with inhibition means of 4 and 3.33 mm, respectively (Table 1). The two microbial agents were comparable to Virtuoso, the standard treatment, with a mean of 4.67 mm. Meanwhile, PCN-2011-005 with inhibition mean of 3.03 mm was similar to PCN-2011-004, Isolate 158 and PCN-2011-002. No significant differences were observed among the following treatments: sterile distilled water, Isolate 73, STR-2011-001, Isolate 94, Isolate 31, Isolate 131, PCN-2011-003 and cuprous oxide as they did not actively inhibit the growth of the pathogen. The two isolates comparable to Virtuoso can be manipulated to either equal the antibiotic potency of Virtuoso or to overcome the latter's diffusion rate, *in-vitro*.

Antibiosis, the antagonism resulting from the production by one microorganism

Table 1. Efficacy of the different microbial antagonists, Virtuoso and cuprous oxide on the growth of *X. campestris pv. vitians*

Treatment	Actual Mean	Transformed Mean
Sterile Distilled water	0.00^{d}	0.71^{d}
PCN-2011-004(*Bacillus sp.*)	4.00^{ab}	4.00^{ab}
PCN-2011-005 (*Bacillus sp.*)	2.33^{c}	2.33^{c}
Isolate 73 (*B. pumilus*)	0.00^{d}	0.71^{d}
PCN-2011-002 (*Bacillus sp.*)	3.03^{bc}	3.03^{bc}
STR-2011-001 (*Bacillus sp.*)	0.00^{d}	0.71^{d}
Isolate 94 (*Flavobacterium sp.*)	0.00^{d}	0.71^{d}
Isolate 31 (*Bacillus sp.)*	0.00^{d}	0.71^{d}
Isolate 131 (*B. pumilus*)	0.00^{d}	0.71d
Isolate 158 (*Pseudomonas sp*.)	3.33^{abc}	3.33^{abc}
PCN-2011-003 (*Bacillus sp.*)	0.00^{d}	0.71^{d}
Virtuoso (*B. subtilis*)	4.67^{a}	4.33^{a}
Cuprous oxide	0.00^{d}	0.71^{d}

of secondary metabolites toxic to other microorganisms, is a form of microbial antagonism wherein direct interaction between two microorganisms shares the same ecological niche (Alabouvette *et al*., 2006). This derived mechanism accounts for the formation of clear zones between the pathogen and the BCA's. Further, Alabouvette *et al* stated that antibiosis is a very common phenomenon responsible for the activity of many BCA's such as fluorescent *Pseudomonas* and *Bacillus spp.*

Fluorescent pseudomonads owe their fluorescence to an extracellular diffusible pigment called pyoverdin (Pvd) or pseudobactin. This pigment has high affinity for Fe^{3+} ions and is a siderophore (iron-carrier) of the producer strain (Meyer and Abdallah, 1978). In iron depleted media *in vitro,* Pvd-producing *Pseudomonas spp*. inhibited the growth of bacteria with less potent siderophores (Kloepper *et al*., 1980), explaining the small halo produced around the susceptible pathogen. Under certain conditions therefore, Pvd functions as a diffusible bacteriostatic antibiotic.

The mechanism for growth suppression employed by *Bacillus spp*. against *X. campestris pv. vitians* as observed was the production of antibiotics, although the type of antibiotics produced in this case is not known. In fungal diseases, *B. subtilis* produced mycosubtilin against *Pythium aphanidermatum* (Leclere *et al*., 2005) while *B. amyloliquefaciens* produced bacillomycin and fengycin against *Fusarium oxysporum* (Koumoutsi *et al*., 2004). Purification and identification therefore of the toxic metabolites produced by the *Bacillus* species are necessary to understand fully the mechanism of control expressed *in vitro* to be able to modify its structure and improve its durability as this antibiotic expression can be lost over time.

Germination rate, seedling height and vigor index as affected by the different microbial antagonists

Isolate 131 gave the highest germination rate at 97.22% with significant differences to Isolate 158, PCN-2011-003, PCN-2011-005, and PCN-2011-002 at 95.83%, 95.83%, 95.55%, and 91.67% respectively (Table 2). However, the latter isolates were comparable to Isolate 31, STR-2011-001, Isolate 94, Isolate 73, Virtuoso, Cuprous oxide and PCN-2011-004 at 90.27%, 90%, 88.87%, 86.11%, 83.34%, 81.94% and 81.94%. Further, the latter isolates are not significantly different to the control.

Germination test on lettuce variety Tyrol revealed that Virtuoso stimulated germination at 90% while cuprous oxide at 89.25% (Calderon, 2011). Results of which were higher compared to what was obtained in this experiment. Since variables can be manipulated, it can be inferred that the percentage germination expressed by the standard treatments operate on numerical variables as 80-90% germination capacity.

Table 2. Influence of microbial antagonists on the germination and growth of lettuce seedlings

TREATMENTS	Seedling Germination Percentage (%)	Seedling Height (mm)	Seedling Vigor Index
Sterile Distilled water	75.00^{b}	67.07^{d}	5033.40^{d}
PCN-2011-004(*Bacillus sp.*)	81.94^{ab}	82.73^{bcd}	6809.38^{bcd}
PCN-2011-005 (*Bacillus sp.*)	95.55^{a}	90.80^{abc}	8670.02^{ab}
Isolate 73 (*B. pumilus*)	86.11^{ab}	83.13^{bcd}	7178.80^{abcd}
PCN-2011-002 (*Bacillus sp.*)	91.67^{a}	83.13^{bcd}	7630.28^{abc}
STR-2011-001 (*Bacillus sp.*)	90.00^{ab}	102.40^{a}	9219.50^{a}
Isolate 94 (*Flavobacterium sp.*)	88.89^{ab}	88.33^{abc}	7856.47^{abc}
Isolate 31 (*Bacillus sp.)*	90.28^{ab}	82.53^{bcd}	7449.39^{ab}
Isolate 131 (*B. pumilus*)	97.22^{a}	92.33^{ab}	8929.56^{ab}
Isolate 158 (*Pseudomonas sp*.)	95.83^{a}	85.80^{abcd}	8248.61^{ab}
PCN-2011-003 (*Bacillus sp.*)	95.83^{a}	85.80^{abcd}	7838.89^{abc}
Virtuoso (*B. subtilis*)	83.34^{ab}	86.33^{abc}	7166.49^{abcd}
Cuprous oxide	81.94^{ab}	72.87^{cd}	5960.53^{cd}

The ability of cuprous oxide to induce germination rate could be attributed to the presence of growth promoting hormones such as IAA, auxins, cytokinins, and gibberellins added to its over-all ingredient. Calderon (2011) citing an article from the agribusiness week stated that copper is an essential microelement needed by plants to promote seed production and formation. Maneb, for example, induces germination of seeds due to the presence of Zn as one of its ingredients. BCA's also produce Indole Acteic Acid (IAA), auxins, cytokinins and gibberellins to promote growth. When Ahmad, *et al* (2006) screened free-living rhizospheric bacteria for their multiple plant growth promoting activities, *Pseudomonas* and *Bacillus* produced IAA, along with Azotobacter isolates.

In the determination of the effect of the biocontrol agents on the seedling height of lettuce, STR-2011-001 promoted seedling growth at 102.40 mm, being the highest among the bacterial agents, followed by Isolate 131, PCN-2011-005, Isolate 94, Virtuoso, Isolate 158, PCN-2011-003, Isolate 73, PCN-2011-004, Isolate 31, cuprous oxide and distilled water at 92.33, 90.80, 88.33, 86.33, 85.80, 85.79, 83.13, 83.13, 82.73, 82.53, 72.87 and 67.07 mm, respectively.

Statistically, STR-2011-001 was comparable to Isolate 131, PCN-2011-005, Isolate 94, Virtuoso, Isolate 158, and PCN-2011-003 and significantly different to PCN-2011-002, Isolate 73, PCN-2011-004, Isolate 31, cuprous oxide, and the control. These reactions indicate that PGPR capacity of microbial agents does not necessitate that germination stimulation and growth promotions are closely linked characteristics. One biocontrol agent may stimulate germination; one may promote growth, while one may have both. Isolate 131 is an example.

In the seedling vigor index tests, STR-2011-001 gave the highest index at 9219.50, however it was comparable to Isolate 131, PCN-2011-005, Isolate 158, Isolate 94, PCN-2011-003, PCN-2011-002, Isolate 31, Isolate 73, and Virtuoso at 8929.56, 8670.02, 8248.61, 7856.47, 7838.89, 7630.28, 7449.39, 7178.80, 7166.49 and differed significantly from PCN-2011-004, cuprous oxide and the control with respective indexes of 6809.38, 5960.53 and 5033.40.

Note that *Pseudomonas sp.* (Isolate 158) gave an index of 8248.60. In the sensitivity screening, its antibiotic behavior was linked to its production of siderophores. Schroth *et al* (1982) postulated that the resulting siderophore hypothesis exert their plant growth promoting activity by depriving pathogens of iron. An example cited by Haas and Defago (2005) was the suppression of *Fusarium* wilt and take-all, by *P. putida* strain B10 but this suppression was lost when the soil was amended with Iron; a move which repressed siderophore production in this strain.

To follow, PGPR can also protect a crop against phyto diseases. Harman *et al* (2004) stated that some biocontrol PGPR elicit a phenomenon known as Induced Systemic Resistance (ISR) in the host plant. ISR is an indirect phenomenon expressed by any plant in reaction to chemical molecules from natural or synthetic origins (Alabouvette *et al*., 2005) which were assumed to be produced by the *Bacillus* and *Pseudomonas* strains utilized in this study. For example, in *Pseudomonas* strains, a combination of pyocyanin and pyochelin seems to be most effective for inducing resistance in tomato (Audenaert *et al*., 2002). The plant growth stimulating volatile 2,3-butanediol that is found in *Bacillus spp.* can also initiate ISR (Ryu *et al.*, 2004).

Calderon (2011) revealed that bacterial isolate 9 induced germination at 95.5%, comparable to Virtuoso and *Trichoderma* with treatment means of 91.5% and 90% respectively. It also promoted seedling height with means of 48.60 mm although not comparable to *Trichoderma* and Virtuoso with treatment means of 61.56 mm and 54.31 mm respectively. Interestingly, bacterial isolate 9 reduced black leaf spot severity with a rating of 1.92, in comparison to the control (3.08) following Virtuoso at 1.83 rating. This could illustrate the effect of PGPR on ISR of lettuce against *X. campestris pv. vitians.* However, it should be noted that ISR and other modes of action are not mutually exclusive; ISR might only exert a complementary effect to microbial antagonism (Alabouvette *et al*., 2005).

Collectively, the results show two promising biological control agents with the capacity to suppress growth of *X. campestris pv. vitians in-vitro*, stimulate germination and promote seedling growth. These are PCN-2011-005 and Isolate158 as shown by table 3 in comparison to the other standard treatments.

Statistically, Virtuoso produced the highest mean of inhibition at 4.33 and was significantly different from Isolate 158, PCN-2011-005, cuprous oxide and the control. Isolate 158 and PCN-2011-005 were however not significantly different from each other.

Table 3. Efficacy of promising microbial antagonists on the growth of *X. campestris pv. vitians* and on the germination, seedling height and seedling vigor of lettuce

TREATMENTS	Inhibition Zone (mm)	% germination	Seedling height (mm)	Seedling vigor index
T_0 – SDW	0.00^{c}	75.00^{b}	67.07^{c}	5033.40^{c}
T_2 – PCN-2011-005 (*Bacillus sp.*)	2.33^{b}	95.55^{a}	90.80^{a}	8670.02^{a}
T_3 – Isolate 158 (*Pseudomonas sp.*)	3.33^{b}	95.83^{a}	85.80^{ab}	8248.61^{ab}
T_4– Virtuoso (*B. subtilis*)	4.67^{a}	83.34^{ab}	86.33^{ab}	7166.49^{abc}
T_5 – Cuprous oxide	0.00^{c}	81.94^{ab}	72.87^{bc}	5960.53^{bc}

In terms of percentage germination, Isolate 158 was the highest at 95.83. This was however not significantly different from PCN-2011-005. Both treatments were however comparable to Virtuoso and cuprous oxide. In terms of seedling height, PCN-2011-005 produced the highest mean at 90.80, comparable to Isolate 158 and Virtuoso but significantly different from distilled water and cuprous oxide. Cuprous oxide was however comparable to Isolate 158 and Virtuoso. In terms of seedling vigor, PCN-2011-005 produced the highest mean at 8670.02, comparable to Isolate 158 and Virtuoso but significantly different from distilled water and cuprous oxide. Cuprous oxide was however comparable to Isolate 158 and Virtuoso.

Note however, that many effective biocontrol PGPR elicit ISR without necessarily producing antibiotics (Zhender *et al*., 2001; Ongena *et al*., 2004; and Ton *et al*., 2002). This could be true to both STR 1 and Isolate 131.

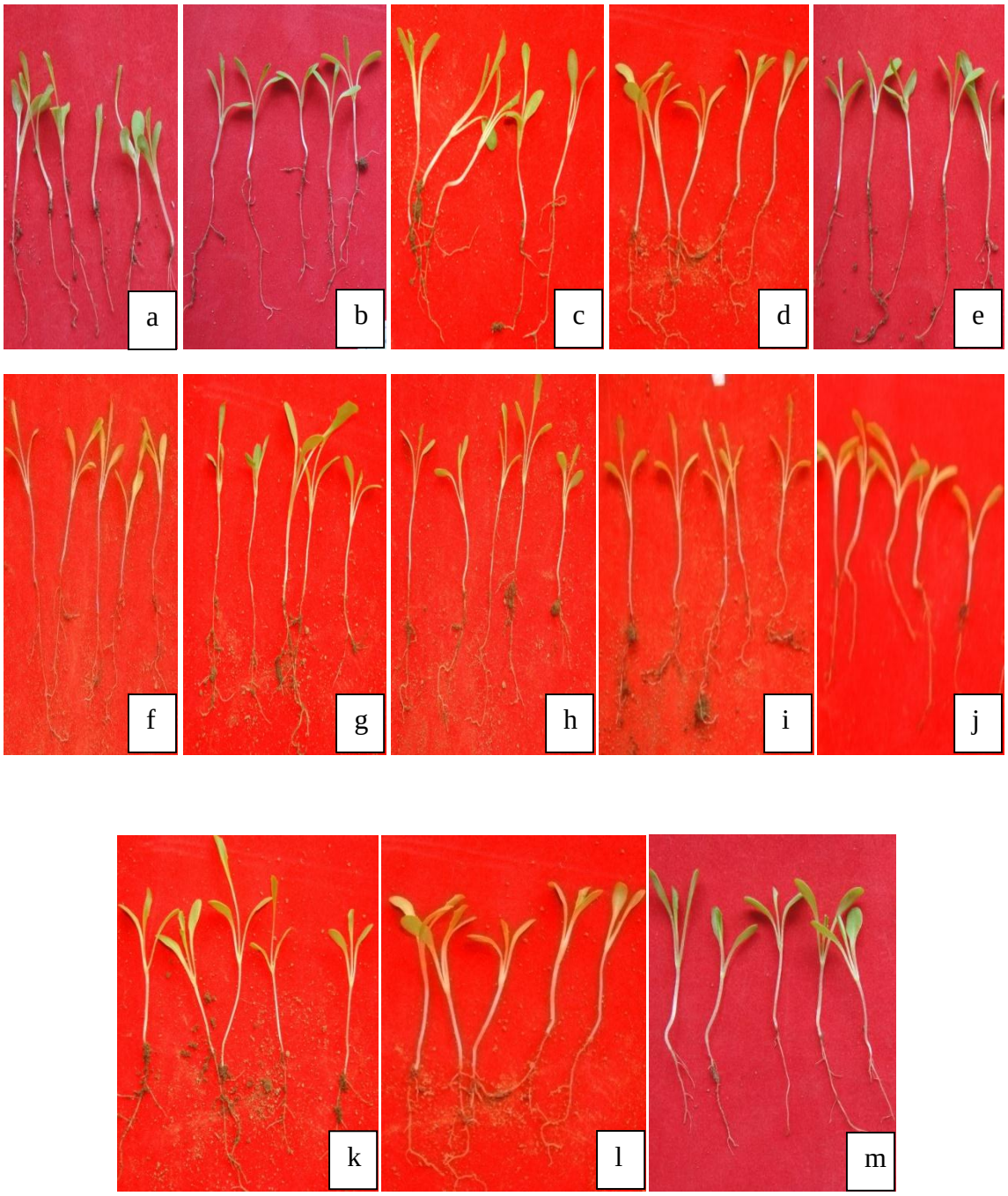


Fig 1. Effect of microbial antagonists on the height of lettuce seedlings: a. Sterile Distilled Water; b. PCN-2011-004; c. PCN-2011-005; d. Isolate 73; e. PCN-2011-002; f. STR-2011-001; g. Isolate 94; h. Isolate 31; i. Isolate 131; j. Isolate 158; k. PCN-2011-003; l. Virtuoso; m. cuprous oxide.

Sensitivity screening of *X. campestris pv. vitians* to different organic-based products at different concentrations

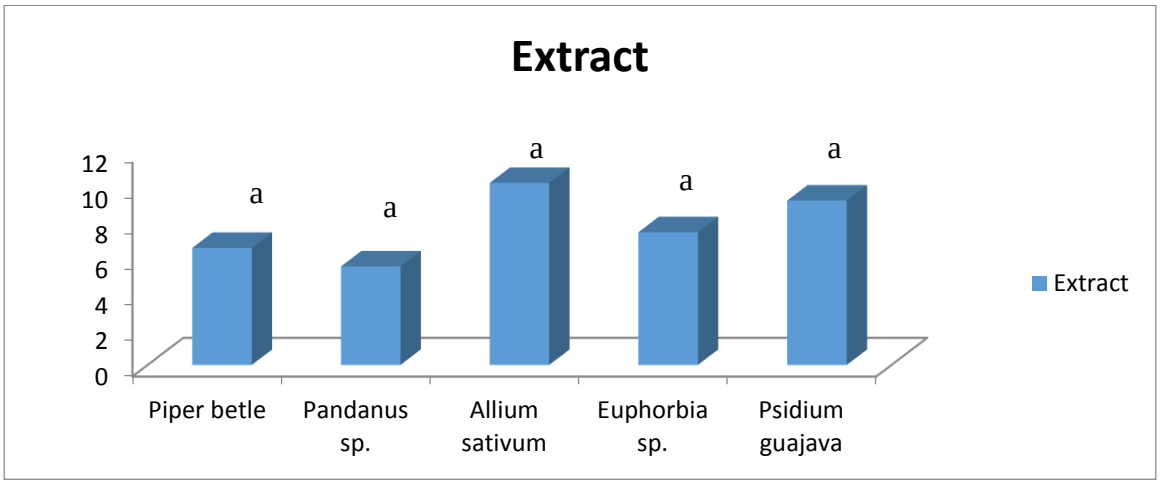


Fig 2. Efficacy of extracts against *X. campestris pv. vitians*

Statistically, no significant difference was obtained from the means of the data analyzed, but numerically, *A. sativum* was the best extract at 10.21, followed by *P. guajava*, *Euphorbia sp.*, *P. betle*, and *Pandanus sp.*, at 9.22, 7.44, 6.56, and 5.52 respectively. The greater antimicrobial activity of *A. sativum* could be attributed to the compound allicin, one of the active principles of freshly crushed garlic homogenates. Allicin has a variety of broad spectrum antimicrobial activities. In its pure form, it was found to inhibit Gram negative bacteria causing human diseases (Ankri and Mirelman, 1999). Lirio *et al* (1999) reported that garlic was able to inhibit the growth of *X. campetsris pv. campestris*, *in-vitro*. The main antimicrobial effect of allicin is due to its chemical reaction with thiol groups of various enzymes, e.g. alcohol dehydrogenase, thioredoxin reductase, and RNA polymerase which can affect essential metabolism of cysteine proteinase activity involved in the virulence of some bacteria (Ankri and Mirelman, 1999). Its effect on *X. campestris pv. vitians* is one concern raised during this study; although, Cavallito and Bailey (1945) reported that inhibition of thiol-containing enzymes in the microorganism by the rapid

reaction of the thiosulfanates with thiol groups was assumed to be the main mechanism involved in the antibiotic effect. It is reasonable to conclude, therefore, that the wide spectrum antimicrobial effects of allicin are due to the multiple inhibitory effects they may have on various thiol-dependent enzymatic systems (Ankri and Mirelman, 1999).

Berdy *et al* 1982 and Caceres *et al* 2005 attributed the antibiotic effect of *P. guajava* to 2 compounds, guajaverin and psidiolic acid against bacteria. Agarwal *et al* (2012) postulated that the antibacterial activity of leaves of *P. betle* against gram positive and gram negative bacteria is due to the presence of metabolic toxins or broad spectrum antimicrobial compounds. *Euphorbia sp*. and *Pandanus sp*. also have antibiotic compounds responsible for the inhibition zones formed around the agar wells against *X. campestris pv. vitians*. Generally, plants elicit a certain chemical as a form of defense mechanism against an invasion by a foreign material.

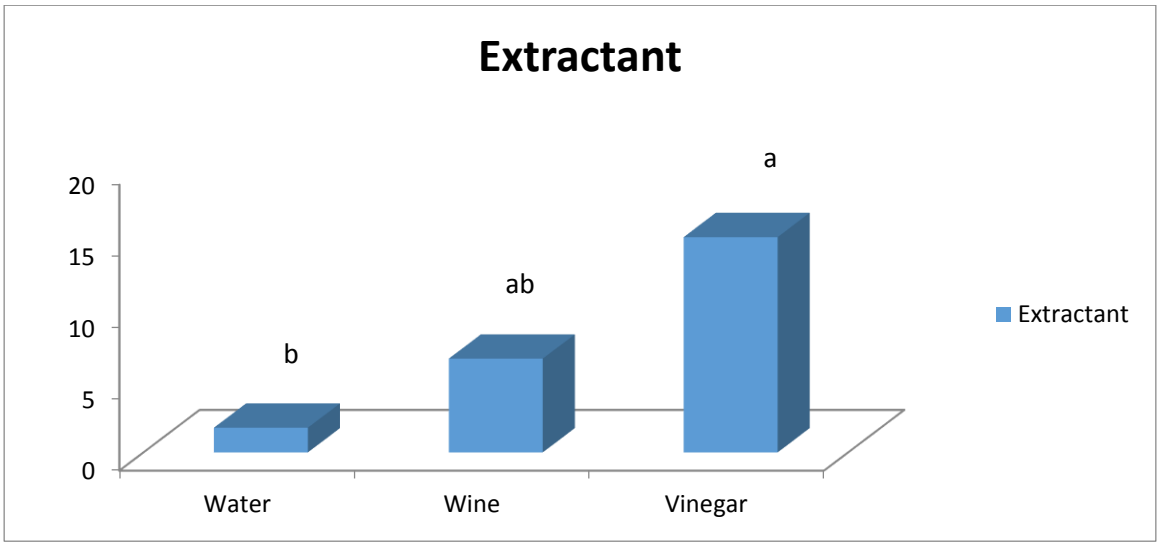


Fig 3. Efficacy of water, wine and vinegar as organic solvents to extract phytochemical compounds from the plant materials

Vinegar showed the highest mean at 15.07, followed by wine and water with means of 6.57 and 1.73, respectively (Fig. 3). Statistically, vinegar was the most effective but this

was comparable to wine and differed significantly with water. Vinegar contains acetic acid, a form of ethanol responsible for the alcohol content of the vinegar. Wines in particular may contain either ethanol or methanol. Alcohols are good solvents for the extraction of phytochemicals situated within the plant cells as they are more efficient in cell wall degradation causing polyphenols to be released from cells. The decrease in activity of aqueous extract (water) can be ascribed to the enzyme polyphenol oxidase, which degrade polyphenols in water extracts whereas in methanol and ethanol (alcohols) they are inactive (Lapornik *et al*., 2005). Reasons as to why wine is lower than vinegar, although they have alcohols, will be discussed further in the phytochemical analysis.

Fig 4 illustrates the effect of the concentration on the efficacy of the extracts.

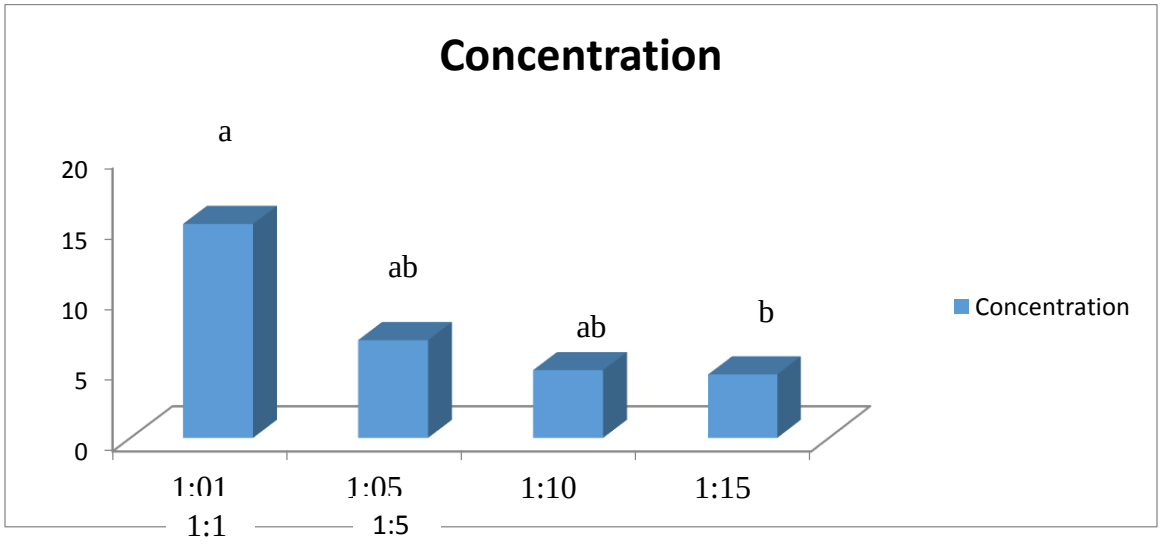


Fig 4. Effect of concentrations on the efficacy of plant extracts against *X. campestris pv. vitians*

Apparently, concentration 1:1 was the most effective but comparable to 1:5, 1: 10 and 1:15 with respective means of 15.14, 6.93, 4.80 and 4.29. The inference therefore is that, as the dilution of solvents to the plant materials is increased, the efficacy of the

concentrations against *X. campestris pv. vitians* decreases. Vis-à-vis, as the dilution decreases, the efficacy of the concentrations increases.

On the other hand, no significant interaction was observed among the three factors, namely: extract, extractant and concentration.

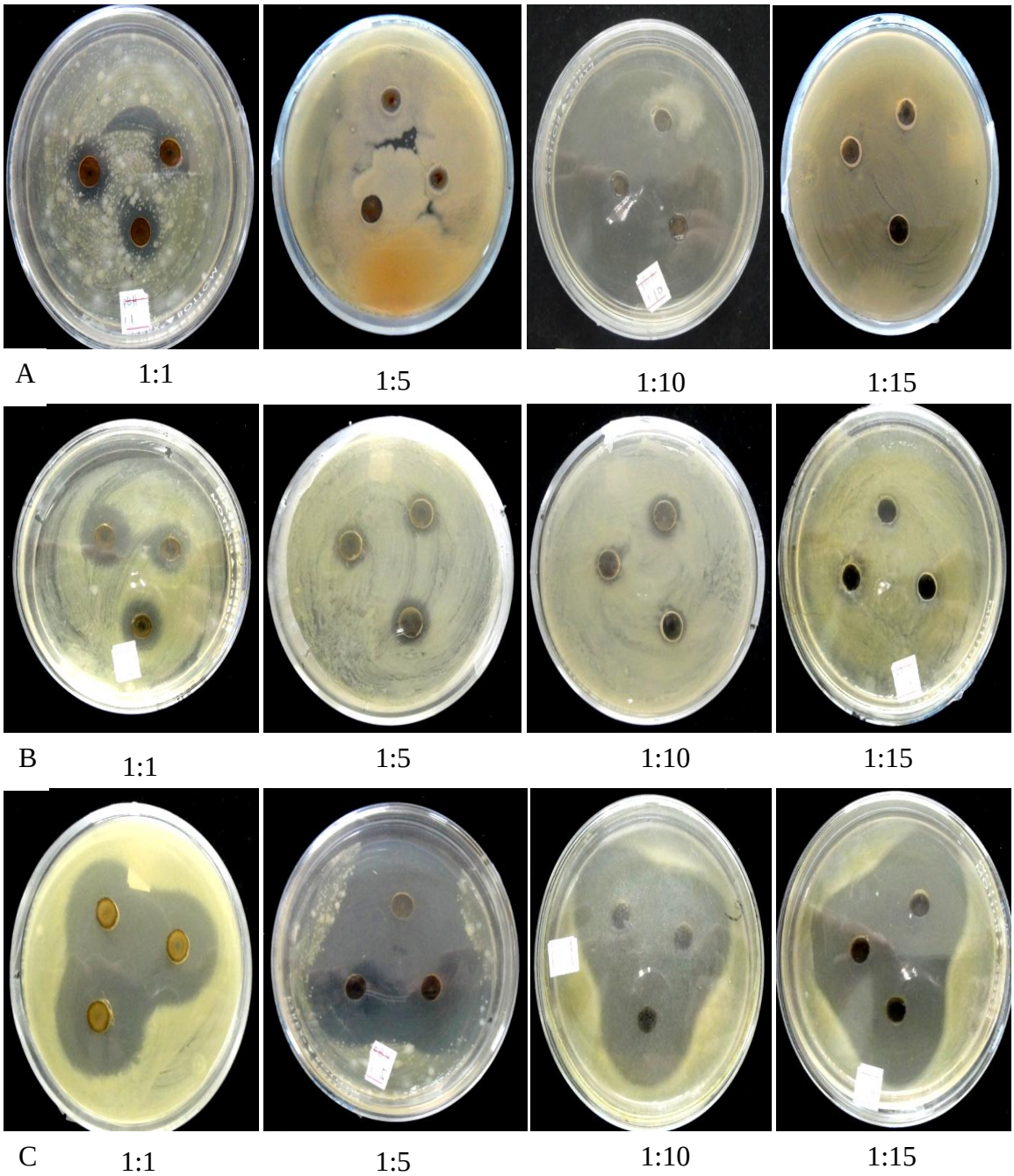


Fig 5. Effect of different extracting solvents and concentrations on the efficacy of *P. betle* against *X. campestris pv. vitians*: A. Water, B. Wine, C. Vinegar

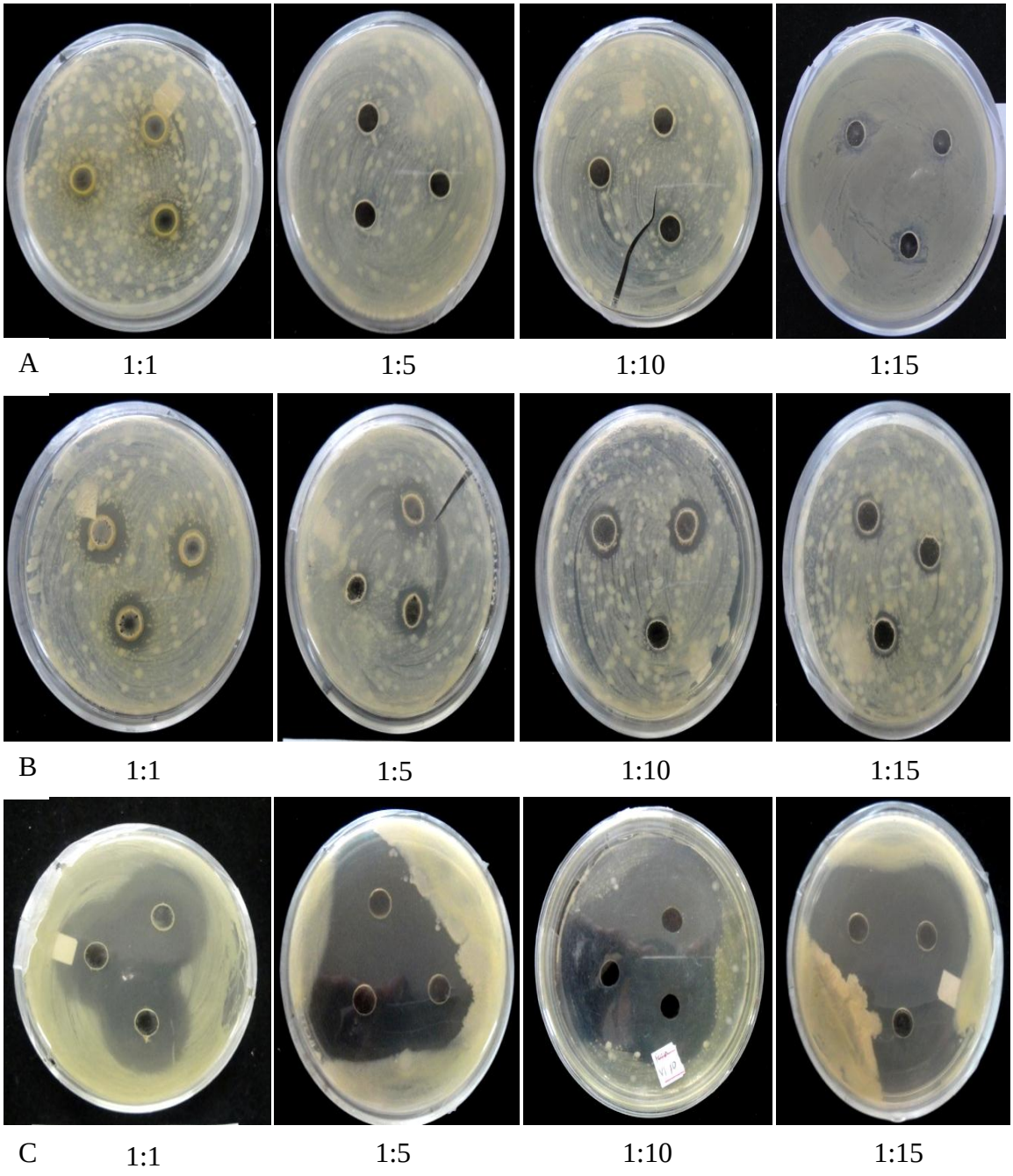


Fig 6. Effect of different extracting solvents and concentrations on the efficacy of *Pandanus sp.* against *X. campestris pv. vitians*; A. Water, B. Wine. C. Vinegar

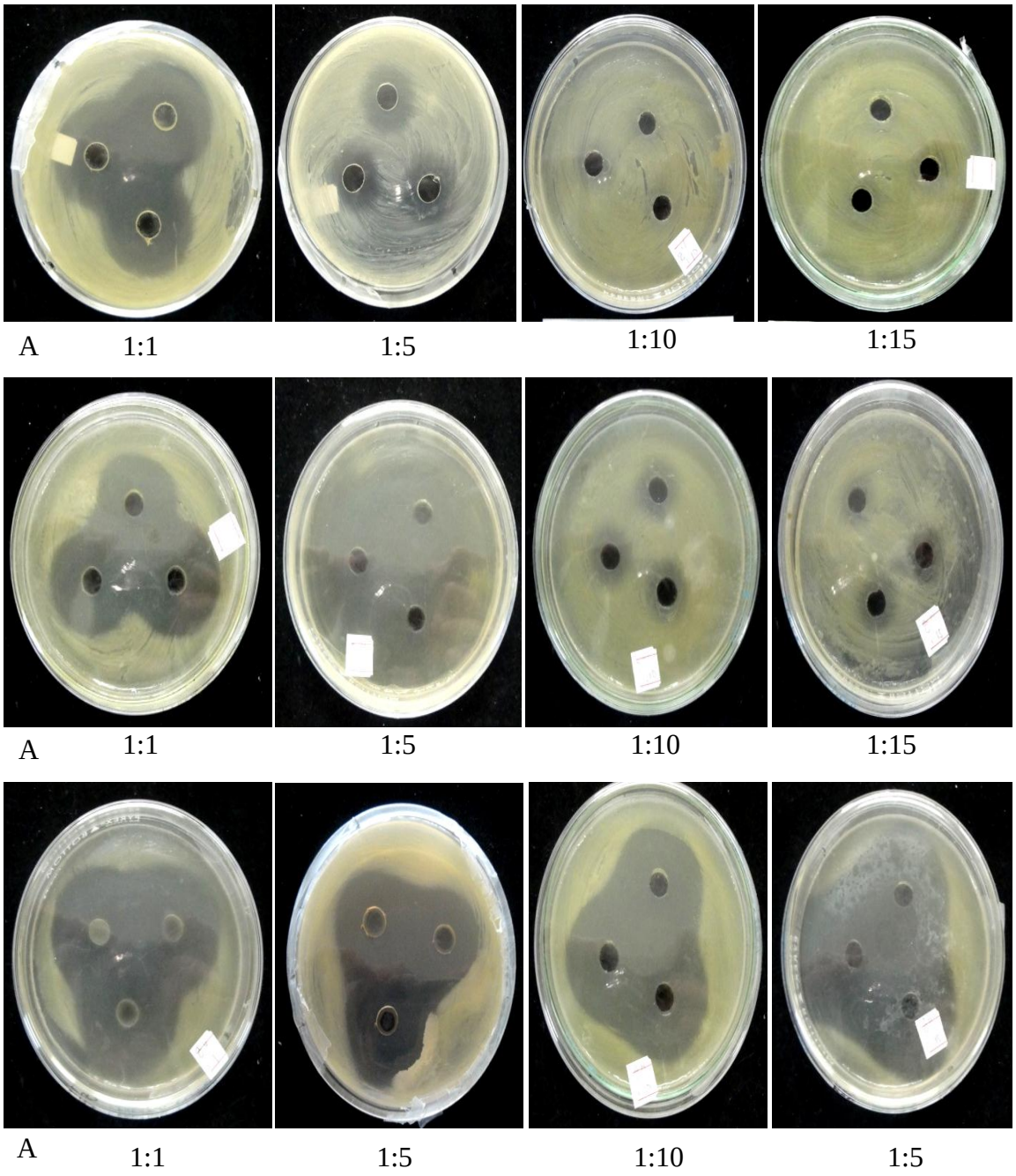
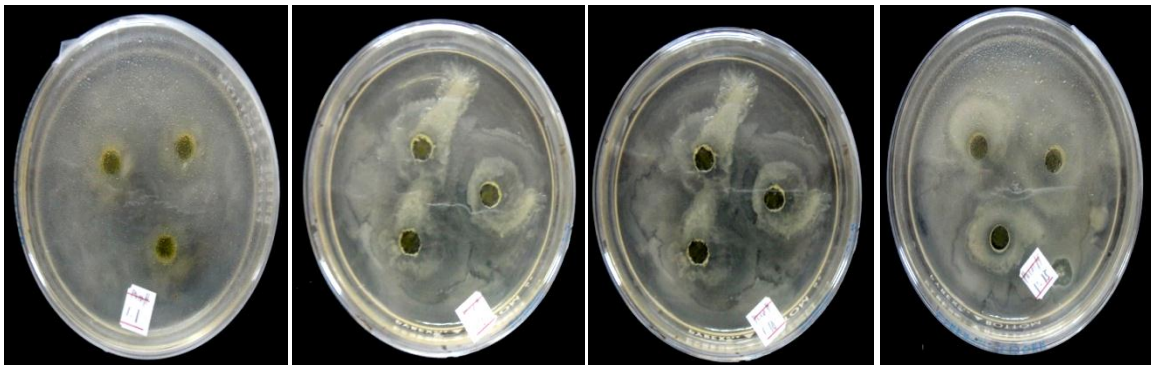


Fig 7. Effect of different extracting solvents and concentrations on the efficacy of *A. sativum* against *X. campestris pv. vitians*; A. Water, B. Wine, C. Vinegar



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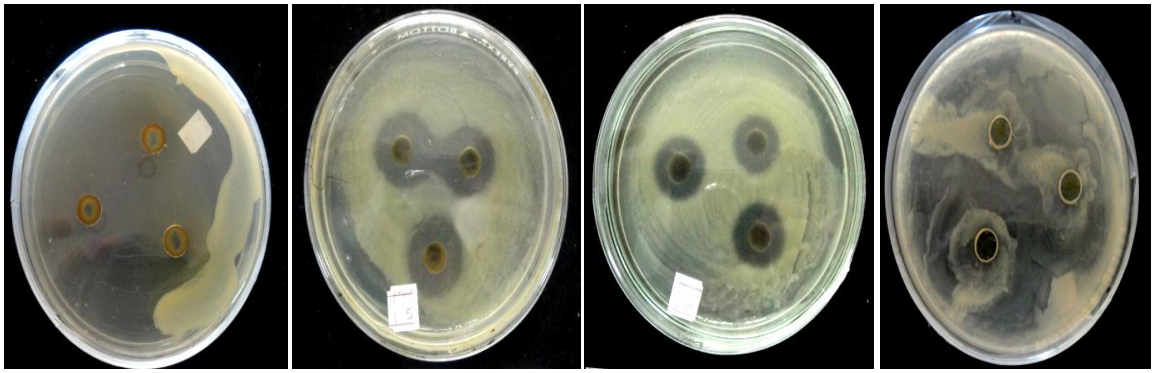
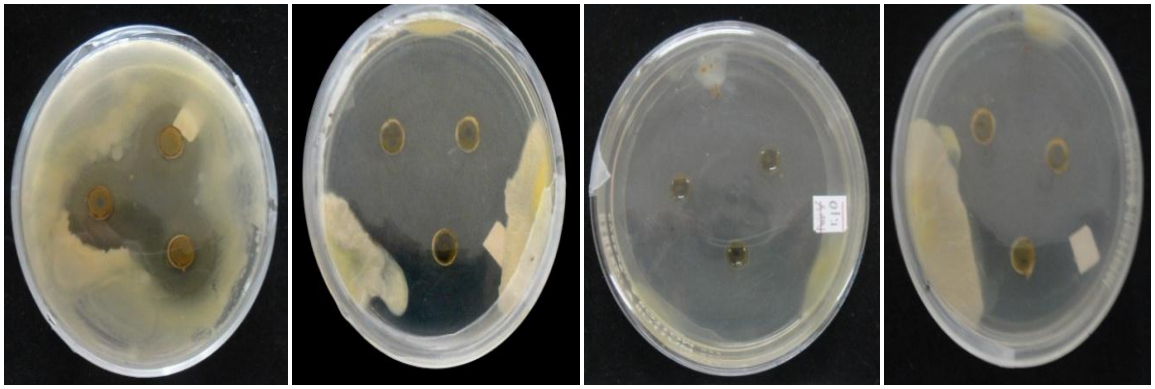
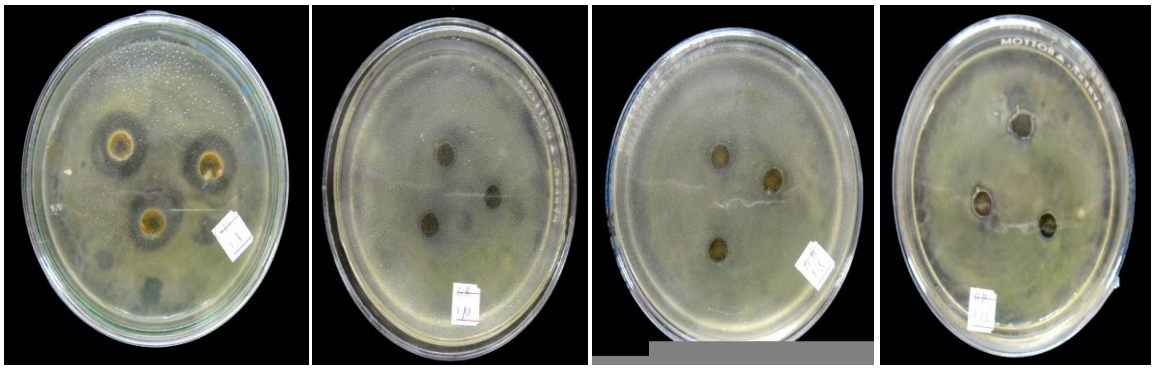


B 1:1 1:5 1:10 1:15



C 1:1 1:5 1:10 1:15

Fig 8. Effect of different extracting solvents and concentrations on the efficacy of *Euphorbia sp.* against *X. campestris pv. vitians*; A. Water, B. Wine, C. Vinegar



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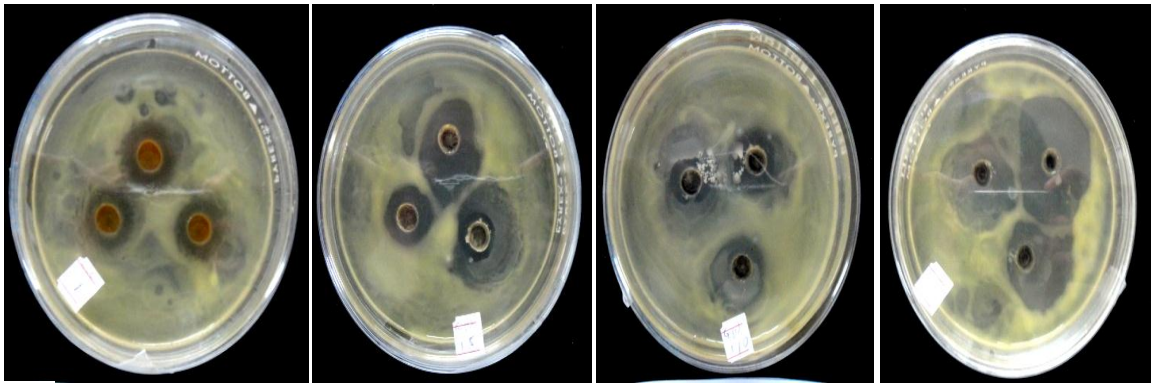

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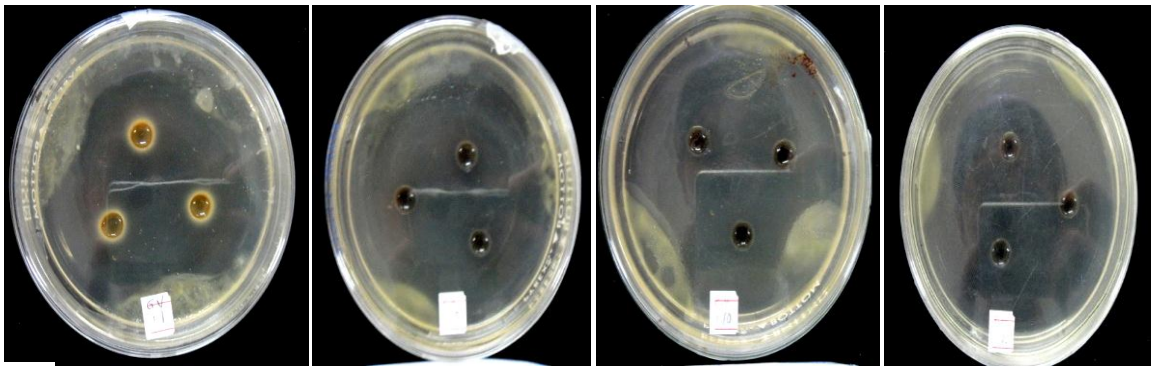

A 1:1 1:5 1:10 1:15

Fig 9. Effect of different extracting solvents and concentrations on the efficacy of *P. guajava* against *X. campestris pv. vitians*; A. Water, B. Wine, C. Vinegar

Phytochemical analysis of the different plant extract - extractant combination

Extensive research has been performed worldwide and important evidences were collected to show the immense potential of plants used in various traditional therapeutic systems (Caunii *et al*., 2005). Many scientists nowadays are exploiting this complex line of systems to answer the problems on resistance exhibited by pathogens to the existing antibiotics. The exploitation of the botanical world begins with the understanding of the phytochemicals present in the plant with specific concern to the bioactive compounds in the plant materials tested against *X. campestris pv. vitians*.

The phenolic compounds present in extracts of plants are always a mixture of different classes of phenols selectively soluble in the solvents (Caunii *et al*., 2005). Successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure (Hirun *et al*, 2012). The solubility of polyphenols depends mainly on the hydroxyl groups, the molecular size and the length of the hydrocarbon chain. The results of the phytochemical analysis from the different extract-extractant combination are shown below:

Table 4. Physiologically active constituents present in the different plant extracts using different extracting solvents

TESTS	RESULTS										CONSTITUENTS
	Pv	Pw	Ph	Pv	pw	gv	gw	gh	Ev	Ew	Gv	
1. Detection of Alkaloids												
1.1Mayer's Test	-	-	+	+	+	-	-	-	+	+	-	Alkaloids
1.2Dragendorff's Test	-	-	-	+		-	+	-	+	+	-	Alkaloids
2. Detection of glycosides												
2.1Modified Borntrager's Test	-	-	-	-	+	-	-	-	-	-	-	Anthraquinone glycoside
3. Detection of Phytosterol												
3.1 Cupric Acetate Test	-	-	-	-	-	+	+	+	-	-	-	Diterpenes
3.2 Salkowski's Test	-	+	+	-	-	+	-	+	-	-	-	Triterpenes
4.Detection of Phenolic compounds	+	+	+	-		-	-	-	-	-	-	Phenolics
4.1 Ferric Chloride Test	-	-	-	+	-	-	-	-	+	-	+	Tannins
4.2 Gelatin Test												
5. Detection of Flavonoids												
5.1 Alkaline Reagent Test	+	+	+	-	+	-	-	-	+	+	+	Flavonoids
5.2 Lead Acetate Test	+	+	+	-		+	+	+	+	+	+	Flavonoids

Physiologically, investigations on the phytochemical analysis of the plant extracts revealed the presence of bioactive and phenolic compounds such as alkaloids, diterpenes, triterpenes, flavonoids, and phenolics, tannins and anthraquinones. These bioactive constituents act as antimicrobial compounds. Quinones bind to adhesins, complex with cell wall and inactivate enzymes. Flavonoids bind to adhesins, and complex with cell wall. Polyphenols and tannins bind to adhesins, inhibits enzyme production, deprives substrate, complexes with the cell wall, disrupts membrane and metal ion complexation. Terpenoids

and essential oils disrupt membrane. Alkaloids intercalate into cell wall and DNA and inhibit release of autocoids and prostaglandins (Tiwari *et al*, 2011).

Results revealed that water can extract the following compounds: alkaloids diterpenes, triterpenes and flavonoids while wine can extract the following compounds: triterpenes, diterpenes and flavonoids. Vinegar, on the other hand, can extract diterpenes and triterpenes, phenolics and flavonoids (Table 4). Since wine and vinegar contain a significant amount of alcohol, it is assumed that bioactive compounds such as alkaloids present in the solutions were changed into another derivative as a result of the organic reactions formed by the alcohols present in the solvents, admonishing their greater antibacterial activity in combination with the extracts as compared to water. Campbell and Smith (2000) stated that when an alcohol reacts with a hemiacetyl group, the resulting compound becomes glycoside. This was probably the phenomenon responsible for the presence of anthraquinone glycoside in *Pandanus sp*. and wine combination. Plant extracts from organic solvents have been found to give more consistent antimicrobial activity compared to water extract because water soluble flavonoids (mostly anthocyanins) have no antimicrobial significance and water soluble phenolics are only important as antioxidant compounds (Das *et al*., 2010). Further, the capacity of the alcohol constituents of both vinegar and wine to polarize the inner cellular membranes of the plants and extract more active constituents is a possible explanation to why water and extracts alone cannot exhibit greater antibacterial activity.

It is also interesting to note that although wine extracted more phytochemicals than vinegar as in the case of *P. betle* whereby *P. betle* + wine extracted triterpenes, vinegar was statistically proven to be the best extractant based from the inhibition zones measured. Vinegar probably extracted a higher amount of phytochemical compounds than wine.

SUMMARY, CONCLUSION AND RECOMMENDATIONS

Summary

The study aimed to; 1. investigate the *in-vitro* activity of ten (10) microbial antagonists against *X. campestris pv. vitians*, 2. determine the effect of each microbial antagonists on the germination and seedling vigor of lettuce seedlings, 3. investigate the *in- vitro* activity of five (5) aqueous plant extracts with three (3) different extracting solvents at different concentrations against *X. campestris pv. vitians* and 4. identify the phytochemicals using different extracting solvents.

Results of the *in-vitro* activity of ten bacterial antagonists against *X. campestris pv. vitians* revealed the significant inhibitory effect of PCN-2011-004 on the growth of the pathogen. This was comparable to the standard biofungicide, Virtuoso and Isolate 158. Although lower in efficacy, PCN-2011-002 and PCN-2011-005 also effected a growth suppression of *X. campestris pv. vitians*.

The highest germination was noted in Isolate 131. However, this was comparable to all the microbial antagonists tested and the standard treatments. The lowest percentage germination was obtained from the untreated seeds.

The highest mean height was noted in STR-2011-001 treated seeds. Nevertheless, this did not significantly differ from Virtuoso, Isolate 131, Isolate 94, Isolate 158, PCN-2011-003 and PCN-2011-005. On the other hand, Isolate 73, PCN-2011-002, PCN-2011-004 and Isolate 31 gave significantly shorter seedlings than the above microbial antagonists but differed significantly from the untreated seeds.

In the *in-vitro* activity of the extracts against the growth of *X. campestris pv. vitians*, no significant interaction was observed among the treatments. Nevertheless, *A. sativum*

produced the highest mean followed by *P. guajava*, *Euphorbia sp*., *P. betle* and *Pandanus sp*. Vinegar solvent gave the highest mean but was comparable to wine. However, this was significantly different from water. The most effective concentration was 1:1 with a mean at 15.13, comparable to 1:5 and 1:10 concentrations but differed significantly from concentration 1:15.

Results of the phytochemical analysis revealed that water can extract the following metabolites: alkaloids diterpenes, triterpenes and flavonoids; wine can extract the following metabolites: triterpenes, diterpenes and flavonoids; and, vinegar can extract the following metabolites: diterpenes and triterpenes, phenolics and flavonoids.

Conclusion

In-vitro tests showed that the microbial antagonists (MA's) and organic-based products tested in this preliminary experiment are potential biopesticides against *X. campestris pv. vitians*. The promising microbial antagonists were Isolate 158 and PCN-2011-004, for inhibiting the growth of the pathogen *in-vitro*; STR-2011-001 and Isolate 131 for stimulating the vigor of the lettuce seedlings *in-vivo*; and, Isolate 158 and PCN-2011-005 for inhibiting the growth of the pathogen *in-vitro* and stimulating the growth of the seedlings *in-vivo*, comparable to the standard Virtouso.

A. sativum combined with vinegar at 1:1 concentration is a potential organic-based product against the said pathogen as its antimicrobial activity *in-vitro* in comparison to the other isolates is more potent than that of the other treatments, regardless of which factor they belong. Phytochemical analysis revealed that each organic solvent was able to extract different kinds of phytochemicals from the cells of the leaves. However, in the sensitivity tests, vinegar showed the highest inhibition zone at 1:1 concentration with no interference

from the pH. This indicates that the alcohol content of bignay vinegar can extract more phytochemical compounds and retain their bonds to the organic phytochemicals when unfavorable microchemical reactions occur.

Recommendations

Based from the results, the following are recommended:

1. Purify and identify the antibiotics present in the bacterial isolates showing inhibition zones;

2. Extend the observation period in the assessment of ZOI (zone of inhibition) to know the most effective application and devise a method or protocol of determining ZOI for a period of five to seven days without necessarily affecting the efficacy of BCA's;

3. Conduct compatibility tests between effective microbial agents and plant extracts;

4. Devise a more rapid and easy extraction methods of extracting phytochemicals from the effective plant materials;

5. Conduct greenhouse and field experiments to validate the efficacy of the potential microbial antagonists and organic-based products against *X. campestris pv. vitians* along with other lettuce diseases.

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