

Controlling Maize Seed Fungi Contamination by Using Various Essential Oil Mixtures

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ABSTRACT

*The effect of various mixture of essential oil for control maize seeds fungi were investigated. Seeds were coated with clove oil (CO) and basil oil (BO), clove oil (CO) and peppermint oil (PO) and basil oil (BO) and peppermint oil (PO) at ratio of 1:3, 1:1 and 3:1 were used respectively. The seed-borne fungi from uncoated seed were assayed by Blotter method. 5 seed-borne fungi were found; 100% *Aspergillus flavus*, 17.5% *Aspergillus niger*, 19.5% *Rhizopus sp.*, 6.0% *Penicillium sp.* and 5.5% *Fusarium sp.* The seeds coated with the mixture of CO and BO at the ratio of 1:3, 1:1 and 3:1 showed the inhibition percentage of all fungi which were not statistically different. CO and BO (1:1) showed the best concentrations that control effectively seed-borne fungi with no negative effects on seed vigor and viability.*

Key words: Maize seed, Essential oil, Seed-borne fungi

INTRODUCTION

Maize is an important raw material in the feed industry. The demand of maize seed is increasing yearly but during the processing and storage of maize seeds, fungi infection is often found, which are commonly *Aspergillus* spp. and *Penicillium* spp. (Marin et al., 1998). These fungi were the result in severe losses in yield and quality of seed (Cardwell et al., 1997). Fungicide seed treatments are normally practiced to prevent fungus before planting. However, chemicals seed treatment are now environmental concerned and limited due to carcinogenicity and teratogenicity of these compounds, they are high toxic and their residues remain long term in the environment and production farm (Gonzalez-Rodriguez et al., 2008). Besides that, chemical had a direct effect on the other side for humans and animals (Giavini and Menegola, 2010). The plant essential oils were investigated and used to prevent fungus. The main component in some of these compounds as eugenol, thymol, menthol were effective in inhibiting the mycelium growth, spores and toxin production of fungi (Basilico and Basilico, 1999). Clove (*Eugenia caryophyllata*) attributed eugenol and could against the growth of *Fusarium oxysporum* (Gupta et al., 2011), *F. verticillioides*, *F. proliferatum*, *F. graminearum* (Velluti et al., 2004), *A. flavus*, *A. niger*, *F. equiseti* and *P. chrysogenum* (Kritzinger et al., 2002). The basil essential oil (3,000 ppm) could completely inhibit *Aspergillus flavus*, *A. parasiticus*, *A. ochraceus* and *F. moniliforme* (Soliman and Badaea, 2002), basil essential oil contains estragol (methyl chavicol), linalool, eugenol and methyl eugenol (Bagamboula et al., 2004). Menthol was the composition of peppermint essential oil (Mahmoud et al., 2004). Peppermint essential oil could inhibit the growth of *A. niger*, *Penicillium* sp. and *P. chrysogenum* (Matan

et al., 2009). Chitosan was applied in conjunction with essential oil to improve the effectiveness of anti-fungus, chitosan had the ability to help prevent fungus and also enhanced the germination of seeds (Burrows et al., 2007) Therefore, this study clove, basil and peppermint essential oils with chitosan were selected and used and evaluated for achieve maximum efficiency in applying anti-fungus on maize seed.

MATERIALS AND METHODS

Experiment

The experiment was conducted at the Seed Laboratory, Department of Plant Science and Natural Resources, Faculty of Agriculture, Chiang Mai University. The selected coating materials were clove essential oils, basil essential oils, peppermint essential oil and chitosan lignosulphonate polymer (CLP). Two experiments were conducted as the following:

Experiment 1: Effect of mixed essential oils to control fungi on maize seed and seed quality

The aim of this experiment was to evaluate the mixed essential oils to control fungi and their effects on seed qualities. The experiments were conducted by using Complete Randomized Design (CRD) with 15 treatments and 4 replications. Maize seed (1 kg) were coated with CLP 8 ml and essential oils 2 ml. The combination of essential oils: clove oil (CO) + basil oil (BO), clove oil + peppermint oil (CO+PO) and basil oil + peppermint oil (BO+PO) in various ratios were 0.5:1.5 (1:3), 1.0:1.0 (1:1) and 1.5:0.5 (3:1) respectively. Uncoated seed was represented as control treatment. The type and quantity of the fungus were examined by Blotter method, the standard germination percentage were tested by between paper method and seed vigor determination as accelerated aging technique, germination index, seedling growth rate, seed vigor classification and shoot and root growth rate (AOSA, 2001; ISTA, 2006) were assayed.

Experiment 2: Evaluation of mixed essential oils to control *Aspergillus flavus* by inoculation

A. flavus was isolated from maize seed and was cultured on potato dextrose agar (PDA) and incubated for 7 days. The concentration of spores was adjusted to 1×10^6 spores/ml by diluting in sterile distilled water. Maize seeds were inoculated by spore suspension of *A. flavus* and were coated with CO and BO (1:1) and chitosan was used as binder. The experiment was designed in CRD using 4 treatments with 4 replications. Then examined the growth inhibition of *A. flavus* by Blotter method and maized seed standard germination percentages were determined and recorded by between paper method.

Statistical analysis

The analysis of variance was performed for data analysis and differentiated with a Least Significant Difference (LSD) comparison at the confidential level of 0.05.

RESULTS AND DISCUSSION

Effect of seed treatment on fungi inhibition and seed quality

Five fungi were found after the examination for the type and quantity of the fungus attached to the maize seeds by Blotter method. They were *A. flavus* 100.0 %, *A. niger* 17.5%, *Rhizopus* sp. 19.5%, *Penicillium* sp. 6.0% and *Fusarium* sp. 5.5%. Coating the seed with various essential oils were tested for their effectiveness to control these fungi. The seeds were coated with CO+BO (1:3, 1:1 and 3:1), CO+PO (1:3 and 1:1), BO+PO (3:1) and captan showed the best results inhibition percentage of *A. flavus*. The seed treatments were coated with CO+BO (1:3, 1:1 and 3:1), CO+PO (1:3, 1:1 and 3:1), BO+PO (1:1 and 3:1) and captan could inhibit *A. niger* better than control. The highest performance in *Rhizopus* sp. inhibiting percentage was seed coated with CO+BO (1:3, 1:1 and 3:1) and captan. Seed coated with CLP and CO+PO (3:1) showed the result to low inhibit *Penicillium* sp. The seed were coated with CO, BO, CO+BO (1:3, 1:1 and 3:1), CO+PO (1:1 and

3:1) and BO+PO (1:3, 1:1 and 3:1) were completely inhibited *Fusarium* sp. Seed coated with CO+BO in all ratios showed no significant differences in inhibiting five fungi (Table 1). The results from this experiment supported many reports from other researcher such as Kritzinger et al. (2002) reported that clove essential oil inhibited growth of *A. flavus*, *A. niger*, *F. oxysporum*, *F. equiseti* and *P. chrysogenum* at 500 and 1000 ppm. The eugenol contents 75.40% in clove essential oil that could inhibitory effect on *Aspergillus* section *Flavi* growth rate (Bluma and Etcheverry, 2008). Basil essential oil contains estragol (methyl chavicol) 20.5%, linalool 16.1%, eugenol was only 3.9% and methyl eugenol 8.0% respectively, (Bagamboula et al., 2004) and it could inhibit the growth of *Rhizopus* sp., *A. niger* and *Fusarium* sp. (Ziedan and Farrag, 2008; Hashem et al., 2010).

Table 1. Effect of coating mixed essential oils on fungi inhibition in maize seed.

| Treatment | Concentration (g/ml per seed 1 kg) | Fungi Inhibition ¹ (%) | | | | |
|---------------------|------------------------------------|-----------------------------------|-----------------|---------------------|------------------------|---------------------|
| | | <i>A. flavus</i> | <i>A. niger</i> | <i>Rhizopus</i> sp. | <i>Penicillium</i> sp. | <i>Fusarium</i> sp. |
| 1. Control | - | 0.00 f | 0.00 f | 0.00 f | 0.00 d | 0.00 d |
| 2. Captan | 3.0 | 99.00 a | 94.44 ab | 93.18 a | 82.14 ab | 90.91 ab |
| 3. CLP | 8.0 | 82.00 d | 62.86 d | 66.67 bcd | 58.34 c | 100.00 a |
| 4. CO | 2.0 | 46.50 e | 77.14 c | 65.87 bcd | 83.33 ab | 78.82 c |
| 5. BO | 2.0 | 55.15 e | 52.12 de | 71.73 bc | 80.00 abc | 100.00 a |
| 6. PO | 2.0 | 46.91 e | 47.94 e | 41.19 e | 100.00 a | 81.82 bc |
| 7. CLP+CO+BO | 8.0+0.5+1.5 | 92.50 abc | 100.00 a | 82.02 abc | 92.00 a | 100.00 a |
| 8. CLP+CO+BO | 8.0+1.0+1.0 | 92.50 abc | 100.00 a | 79.55 abc | 100.00 a | 100.00 a |
| 9. CLP+CO+BO | 8.0+1.5+0.5 | 92.50 abc | 100.00 a | 83.62 ab | 100.00 a | 100.00 a |
| 10. CLP+CO+PO | 8.0+0.5+1.5 | 87.50 bcd | 100.00 a | 66.67 bcd | 100.00 a | 90.91 ab |
| 11. CLP+CO+PO | 8.0+1.0+1.0 | 95.50 ab | 100.00 a | 48.88 de | 100.00 a | 100.00 a |
| 12. CLP+CO+PO | 8.0+1.5+0.5 | 93.50 abc | 100.00 a | 63.41 cd | 66.67 bc | 100.00 a |
| 13. CLP+BO+PO | 8.0+0.5+1.5 | 79.00 d | 82.86 bc | 33.73 e | 85.71 ab | 100.00 a |
| 14. CLP+BO+PO | 8.0+1.0+1.0 | 84.50 cd | 91.44 ab | 50.00 de | 100.00 a | 100.00 a |
| 15. CLP+BO+PO | 8.0+1.5+0.5 | 92.50 abc | 94.87 a | 70.46 bc | 92.86 a | 100.00 a |
| F-test | - | ** | ** | ** | ** | ** |
| LSD _{0.05} | - | 4.48 | 5.90 | 9.05 | 10.80 | 4.93 |

¹Mean values within a column followed by the same letter do not differ significantly to t-test at $P \leq 0.05$

Coated seed with PO, CO+BO (1:1), BO+PO (1:3 and 3:1), captan, CLP and control treatments resulted no significant difference in germination percentage. The CLP treatment showed the highest performance in seeds germination of 96% (Table 2). Chitosan many acted as a growth stimulator that had a profound effect on the growth and development of plant tissue (Nge et al., 2006) and seeds coated with solely clove essential oil had the lowest germination percentage of 81%. Kritzinger et al. (2002) reported that none of the thyme, clove and peppermint essential oils showed harmful effects on the germination and emergence of cowpea seeds. Anyhow, there was the report stated that the clove essential oil contains high eugenol and was toxic to seed germination if used it in improper amounts or high concentration (Liu et al., 2006)

Table 2. Quality of maize seeds after coat with mixed essential oil.

| Treatment | concentration (g/ml per seed 1 kg) | Seed quality ¹ | | | | | | | | | |
|---------------------|--|---------------------------|--------|-----------|---------------------------------|---------------------------|------------|---------|------------------------------------|----------------------------------|--|
| | | Germination (%) | AA (%) | GI | SGR (mg/seedling/ 7 days) | Seed vigor classification | | | Shoot (cm/ seedling/ 5 days) | Root (cm/seedling/ 5 days) | |
| | | | | | | High (%) | Medium (%) | Low (%) | | | |
| 1. Control | - | 92 abcd | 80 cde | 20.26 ab | 0.0759 bcd | 31 cd | 52 abc | 11 a | 6.88 fg | 12.44 cd | |
| 2. Captan | 3.0 | 94 ab | 85 ab | 20.56 a | 0.0787 ab | 75 a | 18 g | 2 d | 8.23 abc | 12.38 cd | |
| 3. CLP | 8.0 | 96 a | 82 bcd | 20.39 ab | 0.0781 ab | 51 b | 41 d | 4 cd | 8.84 a | 14.15 a | |
| 4. CO | 2.0 | 81 e | 72 g | 17.19 ef | 0.0646 g | 31 cd | 30 f | 10 a | 5.77 h | 10.76 ef | |
| 5. BO | 2.0 | 91 bcd | 78 def | 18.78 cd | 0.0765 abc | 50 b | 26 f | 10 a | 7.20 efg | 12.49 bcd | |
| 6. PO | 2.0 | 92 abcd | 74 fg | 19.53 bc | 0.0729 cde | 25 d | 57 a | 9 ab | 7.07 fg | 12.32 cd | |
| 7. CLP+CO+BO | 8.0+0.5+1.5 | 91 bcd | 83 abc | 18.32 d | 0.0709 ef | 48 b | 39 de | 4 cd | 7.33 defg | 13.50 ab | |
| 8. CLP+CO+BO | 8.0+1.0+1.0 | 92 abcd | 84 ab | 18.05 de | 0.0662 g | 47 b | 40 d | 6 bc | 8.01 bcd | 13.99 a | |
| 9. CLP+CO+BO | 8.0+1.5+0.5 | 89 d | 81 bcd | 17.19 ef | 0.0662 g | 31 b | 52 abc | 4 cd | 6.62 g | 10.83 ef | |
| 10. CLP+CO+PO | 8.0+0.5+1.5 | 91 bcd | 81 bcd | 18.16 d | 0.0727 cde | 37 c | 45 cd | 5 c | 7.97 bcde | 10.27 f | |
| 11. CLP+CO+PO | 8.0+1.0+1.0 | 91 bcd | 79 cde | 17.91 def | 0.0722 de | 47 b | 31 ef | 10 a | 7.75 cdef | 12.09 d | |
| 12. CLP+CO+PO | 8.0+1.5+0.5 | 90 cd | 76 efg | 17.11 f | 0.0672 fg | 26 d | 55 ab | 5 c | 7.18 fg | 11.55 de | |
| 13. CLP+BO+PO | 8.0+0.5+1.5 | 92 abcd | 81 bcd | 18.30 d | 0.0668 g | 37 c | 48 bc | 5 c | 8.40 abc | 13.32 abc | |
| 14. CLP+BO+PO | 8.0+1.0+1.0 | 91 bcd | 85 ab | 18.21 d | 0.0763 bc | 51 b | 27 f | 10 a | 7.69 cdef | 12.11 d | |
| 15. CLP+BO+PO | 8.0+1.5+0.5 | 93 abc | 86 a | 18.78 cd | 0.0802 a | 45 b | 39 de | 6 bc | 8.57 ab | 13.23 abc | |
| F-test | - | ** | ** | ** | ** | ** | ** | ** | ** | ** | |
| LSD _{0.05} | - | 1.94 | 2.23 | 0.46 | 0.001 | 4.17 | 3.75 | 1.53 | 0.38 | 0.52 | |

¹Mean values within a column followed by the same letter do not differ significantly to t-test at $P \leq 0.05$

AA: Accelerated aging test; GI: Germination index; SGR: Seedling growth rate; High, Medium and Low: Seed vigor classification; Shoot: Shoot growth rate; Root: Root growth rate. Concentration of mixed essential oils; 0.5+1.5: (1:3), 1.0+1.0: (1:1), 1.5+0.5: (3:1)

The seed coated with CO+BO (1:3 and 1:1), BO+PO (1:1 and 3:1) and captan showed no significant differences and the best results in vigor test by accelerated aging technique. The germination index of seed lot coated with captan, CLP and control were 20.56, 20.39 and 20.26 respectively. The seed coated with BO+PO (3:1) provided seedling growth rate better than other coated treatments. When classify the vigorous of the seedling, the seed coated with captan had the high number vigor seedling of 75% followed by CLP and BO+PO (1:1) 51%, BO 50%, CO+BO (1:3) 48%, CO+BO and CO+PO (1:1) 47%, BO+PO (3:1) 45% and CO+BO (3:1) 31% respectively. Coated seed with CLP showed the fastest growing shoot and root growth rate of 8.84 and 14.15 respectively, and were not significant differences with the seed coated with BO+PO (1:3 and 3:1).

Germination and *A. flavus* percentage after using mixed essential oils for control *A. flavus* on maize seed

After inoculated *A. flavus* to the seed, the seed germination percentages dropped from 92% to 80% with significantly differences. The seed germination percentages of mixed essential oils coated seed with CO+BO (1:1) and coated seed after inoculated *A. flavus* to seeds were not significantly differences (Figure 1). It might be due to the too short time of storage before testing. The essential oils effected to fungi that it was low effective to infect seed coat (Basilico and Basilico, 1999). After inoculated *A. flavus* and coated seed with CO+BO (1:1) that *A. flavus* remained 18% higher than seed treatment with CO+BO (1:1) and no effect to their germination percentage.

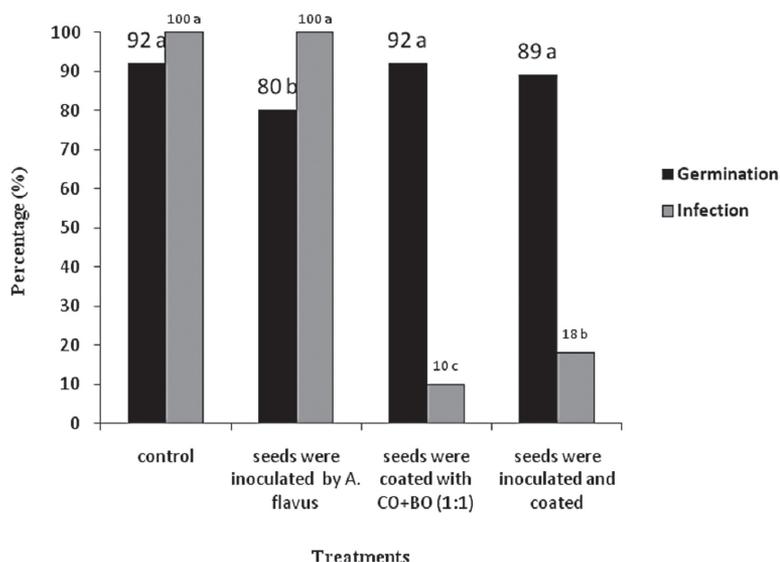


Figure 1. Effect of clove and basil (1:1) essential oils on inhibiting *A. flavus* after inoculate on maize and seed germination percentage.

CONCLUSION

Five seed-borne fungi from uncoated seed were found: *A. flavus*, *A. niger*, *Rhizopus* sp., *Penicillium* sp. and *Fusarium* sp. at the rate of 100.0, 17.5, 19.5, 6.0 and 5.5% respectively. The seeds coated with the mixture of CO+BO at the ratio of 1:3, 1:1 showed the best result in inhibiting (100% *A. niger*; *Penicillium* sp. and *Fusarium* sp.) all fungi. The combination of CO+BO (1:1) resulted best in seed germination, seedling vigor by accelerated aging, seed vigor classification and seedling growth rate which significantly differences than the other treatments. The inoculation *A. flavus* and coated with CO+BO (1:1) showed no effected to seed germination.

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