# Potential of Essential Oils from four Cameroonian Aromatic plants used in Integrated Protection of Stored Products programs

**Leon Azefack Tapondjou[1]*, Verlaine Woguem[1,2], Hilaire Macaire Womeni[2]**

[1]Laboratory of Environmental and Applied Chemistry, Faculty of Science, University of Dschang, PO Box 67, Dschang, Cameroon
[2]Laboratory of Biochemistry of Medicinal Plants, Food Science and Nutrition, Faculty of Science, University of Dschang, PO Box 67, Dschang, Cameroon
*Corresponding author: L. A. Tapondjou (tapondjou2001@yahoo.fr)
DOI 10.5073/jka.2018.463.202

## Abstract

The efficacy of essential oils extracted from fruits of *Piper capense* and *Xylopia parviflora*, and roots of *Echinops giganteus* and *Mondia whitei* were evaluated against *Acanthoscelides obtectus* and fungi isolated from bean seeds in laboratory conditions in Cameroon. The essential oils were extracted by water-distillation and their chemical composition identified by Gas Chromatography-Flame Ionization Detection (GC-FID) and Gas Chromatography-Mass Spectrometry (GC-MS). Toxicity assays of essential oils against *A. obtectus* were carried out by fumigation in which insect pests were exposed fumes of the essential oils, and mortality recorded after 6, 12, and 24 hours. Additionally, the toxicity by contact of the essential oils was evaluated through coating grains with essential oils or impregnating the essential oils onto the filter paper, allowing the insects to physically get in contact with the essential oil, and assessing weevil mortality recorded after 1, 2, 3, and 4 days. The essential oils from *P. capense* and *X. parviflora* consisted mainly of hydrocarbon monoterpenes (56.5% and 50.0% respectively), whereas the essential oils from *E. giganteus* was mostly constituted of sesquiterpenes (94.3%) in which the tricyclic compounds are more abundant. A major compound identified in the essential oil from *M. whitei* was 2-hydroxy-4-methoxy-benzaldehyde (81%). The essential oil from *X. parviflora* was the most effective as contact and fumigant against *A. obtectus,* causing 100% mortality within 1 day at low lethal concentrations. On the other hand, the essential oil from *M. whitei* exhibited the best anti-fungal activity. These essential oils could play an important role in pest protection of stored beans and reduce the risks associated with use of synthetic insecticides especially in low income small holder farming systems.

**Keywords :** Essential oil, Insecticide, Fungicide, biopesticides, Integrated Pest Management

## Background

Bean crops (Phaseolus vulgaris L.) occupy a prominent place in medium and large farming units in Cameroon (Pessoa et al., 2016). Most of the time, the availability of this crop depends on, among other factors, strict quality control, timely harvesting, and appropriate storage. During storage, bean seeds may be destroyed by insects mainly Acanthoscelides obtectus SAY which consume substantial quantities of the beans, and their respiration increases temperature and intergranular humidity which in turn facilitates fungal growth (Rupolho et al., 2006) and production of mycotoxins. Stored grain pest infestation is controlled by various methods amongst which the application of chemical pesticides remains the most effective. However, because of the negative side effects of most synthetic insecticides on environment and human health, alternative control methods are gaining importance. Over the last decade, essential oils from plant origin and other botanicals (plant powders, plant extracts and non-volatile oils) have been developed as potential alternatives for pest control. They are often of low mammalian toxicity, readily biodegradable and pose low danger to the environment if used in small amounts (Regnault-Roger et al., 2002). This research evaluated the insecticidal and fungicidal activities of essential oils from fruits of Piper capense and Xylopia parviflora, and roots of Echinops giganteus and Mondia whitei against Acanthoscelides obtectus and fungi isolated from bean seeds. These plants were selected among others because they are locally available and used as spices in some camerounian traditional foods.

## Materials and methods

*Toxicity assays of essential oils extracted from* Piper capense, Xylopia parviflora, Echinops giganteus *and* Mondia whitei against *the bean weevil (*Acanthoscelides obtectus*)*

Adult insects were obtained from stock cultures maintained in the laboratory and reared on common bean grains. They were kept in a controlled chamber under a 10-h light, 14-h dark photoperiod at 26.08 ± 0.2 °C and 70.8 ± 0.4% relative humidity (RH). After two weeks of oviposition, parent insects were removed by sieving and the glass bottles containing bean were held under the same conditions until the emergence of F1 progeny. For activity testing, one or two days old adult of unsexed insects were used. The different plant materials were collected from Dschang market where they are sold as spices. Essential oils of *Piper capense* and *Xylopia parviflora* fruits, of *Echinops giganteus* and *Mondia whitei* roots were extracted by hydro-distillation and analyzed by Gas Chromatography-Flame Ionization Detection (GC-FID) and Gas Chromatography-Mass Spectrometry (GC-MS). Toxicity assay of essential oils against *A. obtectus* was carried out by fumigation in which insect pests were exposed fumes of the essential oils, and mortality recorded after 6, 12, and 24 hours. Additionally, the toxicity by contact of the essential oils was evaluated through coating grains with essential oils or impregnating the essential oils onto the filter paper, allowing the insects to physically get in contact with the essential oil, and assessing weevil mortality recorded after 1, 2, 3, and 4 days. Percent mortality was calculated using the Abbott correction formula for natural mortality in controls (Abbott, 1925). The Bliss (1938) method based on the regression of the probits of mortalities (Finney, 1971) was used to determine the 50% lethal concentration (LC50) based on the decimal logarithms of the oil concentrations. After the above contact toxicity tests, the remaining living adults were removed, discarded and the glass jars containing beans were kept under the same experimental conditions until the emergence of F1 progeny. The emerged F1 insect's was counted to evaluate the effect of essential oils on the progeny production of *A. obtectus* adults. Percentage of reduction in progeny production was calculated and the damaged seeds were weighed to assess weight loss.

Evaluating antifungal activity of essential oils extracted from Piper capense, Xylopia parviflora, Echinops giganteus and Mondia whitei against different fungi isolated from market bean seeds

Thirty-six samples of bean were collected from three markets in Dschang town, at the rate of 12 from each market. To isolate post-harvest pathogens, 10 seeds of each sample were placed in Petri dishes on potatoes dextrose agar (PDA) for 7 days at 21±2 °C. The pure isolated fungi were morphologically identified according to the documented keys in fungal identification (Champion, 1997; Mathur and Kongsdal, 2003) and were subcultured and stored in a fridge (4 °C) until needed. Among these, 8 filamentous fungi (*Aspergillus flavus, Aspergillus niger, Fusarium oxysporum, Fusarium moniliforme, Fusarium nivale, Fusarium solani, Fusarium crookwellense,* and *Penicillium* sp) were used to evaluate the antifungal activity of essential oils. The antifungal activity of the essential oils was performed following the procedure described (Ngono et al., 2000). The minimal inhibitory concentration (MIC) which inhibits the visible growth of fungi was recorded. For confirmation of the fungistatic or fungicidal activity, all wells showing no visible growth after 7 days were subcultured onto potatoes dextrose agar medium and incubated at 25°C for 10 days and the minimal fungicidal concentration (MFC) was recorded as the lowest concentration where no fungal growth was observed.

## Results

Chemical composition of the essential oils

The essential oils of *P. capense* and *X. parviflora* consisted mainly of hydrocarbon monoterpenes (56.5% and 50.0% respectively), followed by hydrocarbon sesquiterpenes (17.8%) for *P. capense* and oxygenated monoterpenes (20.7%) for *X. parviflora*, whereas *E. giganteus* was mostly constituted of sesquiterpenes (94.3%) in which the tricyclic compounds are more abundant. A major compound, 2-hydroxy-4-methoxy-benzaldehyde (81%) was identified in the essential oil of *M. whitei* (Tab. 1).

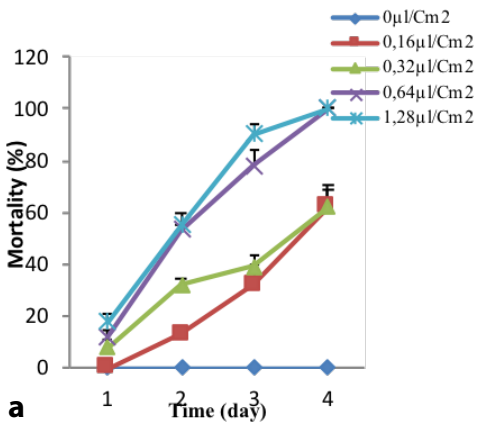
**Tab. 1.** Chemical composition of the essential oil from *Echinops giganteus, Piper capense* and *X. parviflora*.

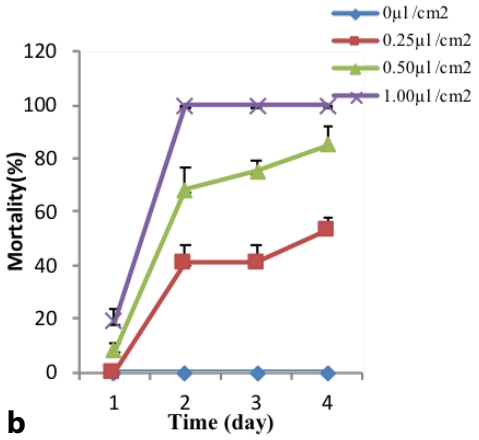
| | Essential oils (%) | | |
|---|---|---|---|
| Compound name | Echinops giganteus | P. capense | Xylopia parviflora |
| 4-hydroxy-4-methyl-2-pentanone | / | / | 0.3 |
| α-thujene | / | 0.6 | 0.1 |
| α-pinene | / | 8.9 | 10.3 |
| camphene | / | 0.6 | 3.3 |
| sabinene | / | 10 | / |
| β-pinene | / | 33.2 | 34 |
| myrcene | / | 0.9 | 0.1 |
| δ-3-carene | / | 0.5 | / |
| p-cymene | / | 0.4 | 1.3 |
| limonene | / | 1.8 | 0.6 |
| 1,8-cineole | / | 0.4 | 1.7 |
| Cis-sabinene hydrate | / | 0.3 | / |
| linalool | / | 1.3 | 0.9 |
| α-campholenal | / | / | 0.2 |
| trans-pinocarveol | / | 0.6 | 5.0 |
| camphor | / | 0.2 | 0.4 |
| pinocarvone | / | 0.1 | 0.4 |
| borneol | / | 0.6 | 0.5 |
| terpinen-4-ol | / | 1.2 | 0.8 |
| p-cymen-8-ol | / | 0.5 | 0.1 |
| α-terpineol | / | 0.6 | 1.0 |
| myrtenal | / | 0.3 | 2.5 |
| myrtenol | / | 0.4 | 4.6 |
| verbenone | / | / | 0.5 |
| isobornyl acetate | / | 2.0 | 1.0 |
| 7-epi-silphiperfol-5-ene | 3,5 | / | / |
| α-cubebene | / | 0.4 | 0.3 |
| cyclosativene | / | 0.2 | 0.5 |
| modheph-2-ene | 3,0 | / | / |
| α-copaene | / | 0.6 | 0.5 |
| silphiperfol-6-ene | 23,0 | / | / |
| α-isocomene | 2,4 | / | / |
| β-cubebene | / | 1.2 | 0.3 |
| β-elemene | / | 1.0 | 0.5 |
| β-isocomene | 2,1 | / | / |
| (E)-caryophyllene | 6,3 | 6.3 | 0.2 |
| β-copaene | / | 0.1 | 1.1 |
| Gamma-elemene | / | 0.3 | / |
| 6,9-guaiadiene | / | 0,2 | / |
| α-humulene | 2,0 | 1.1 | 0.1 |
| (E)-β-farnesene | / | 0.1 | / |
| germacrene D | 0,3 | 3.8 | / |
| β-selinene | / | 0.3 | 0.1 |
| trans-muurola-4(14), 5-diène | / | 0.1 | 0.1 |
| α-muurolene | / | 0.6 | 0.6 |
| silphiperfolan-6-α-ol | 1,0 | / | / |
| cameroonan-7-α-ol | 7,1 | / | / |
| silphiperfolan-7-β-ol | 2,5 | / | / |
| trans-calamenene | / | / | 1.4 |
| δ-cadinene | 0,3 | 0.8 | / |
| hedycaryol | / | / | 1.2 |
| germacrene B | / | 0.6 | / |
| silphiperfolan-6-β-ol | 1,7 | / | / |
| (E)-nerolidol | / | 1.5 | / |
| prenopsan-8-ol | 3,2 | / | / |
| caryophyllene oxide | / | 2.8 | 2.1 |
| presilphiperfolan-8-ol | 22,7 | / | / |
| salvial-4(14)-en-1-one | / | 0.2 | 0.3 |
| humulene epoxide II | / | 0.3 | 0.8 |
| 1,10-di-epi-cubenol | 0,1 | 0.4 | 2.2 |
| caryophhyla-4(12),8(13)-dien-5-ol | / | 0.1 | 0.7 |
| epi-α-muurolol | 0,4 | | |
| cubenol | / | 0.3 | 0.8 |
| α-muurolol | 0,1 | 0.4 | 2.1 |
| α-cadinol | 0,4 | / | / |
| cis-calamenen-10-ol | / | / | 0.3 |
| trans-calamenen-10-ol | / | / | 0.2 |
| eudesma-4(15),7-dien-1-β-ol | / | 0.3 | 1.0 |
| curcuphenol | 0,4 | / | / |
| manoyl oxide | / | / | 1.0 |
| phyllocladene | / | / | 0.2 |

Toxicity of extracted essential oils to Acanthoscelides obtectus

The toxicity of essential oils applied on filter paper was dose-dependent because weevil mortality increased with concentrations of the oils, except for *M. whitei* which did not show any activity against *A. obtectus* (Fig.1). Essential oil extracted from *X. parviflora* fruits was the most effective at all concentrations evaluated. At the highest concentrations (0.31 and 0.47µl/cm2), it caused 100% mortality within the first day, whereas the essential oils from *E. giganteus* and *P. capense* caused weevil mortality after 2 and 4 days exposure, respectively. The LC50 of essential oils extracted from *E. giganteus, P. capense* and *X. parviflora* when used as physical contact poisons against *A. obtectus* were 0.35 µl/cm2, 0.31µl/cm2 and 0.17µl/cm2, respectively.

After 24h, the lowest concentration of all fumigated essential oils had strong toxic action (between 98 and 100% mortality) against *A. obtectus* (Fig. 2). Between 6h and 12h, the mortality induced by all the concentrations was significantly different ($P<0.05$). The LC50 values after 12h were 0.15 µl/cm3, 0.08 µl/cm3 and 0.05µl/cm3 for *M. whitei, P. capense* and *X. parvflora*, respectively. The essential oil from *E. giganteus* was not effective against *A. obtectus* at all the tested concentrations when used as a fumigant. The mortality of *A. obtectus* due to fumigation with essential oils also increased with the dosage of oil and the exposure time. Essential oil *of X. parviflora* was the most effective against A. *obtectus* on beans. Indeed, its highest doses (0.04 and 0.06µl/g) caused 100% mortality on the first day. The LD50 of the essential oils calculated after 2 days of exposure when used as fumigants against *A. obtectus* adults were 0.80µl/g, 0.60µl/g and 0.19µl/g for *M. whitei, P. capense* and *X. parvflora*, respectively.



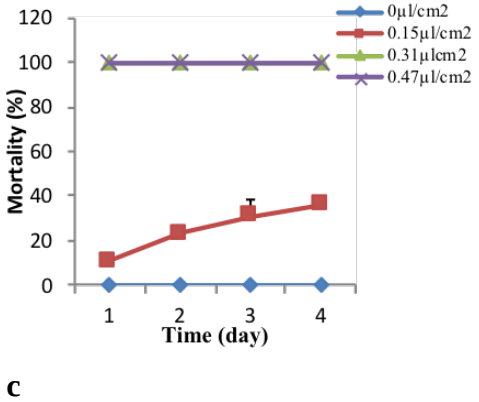
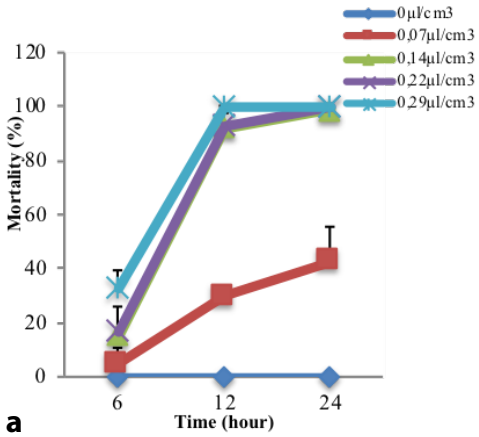
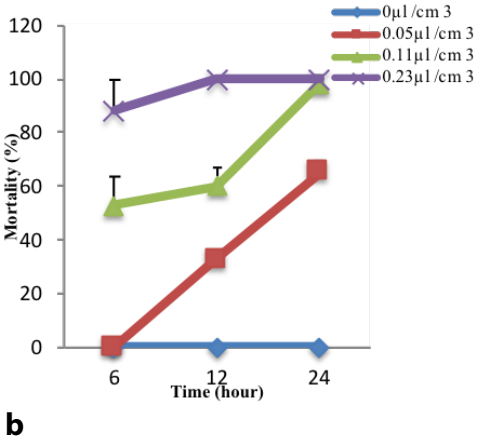




**Fig 1**: Mortality percentage (CM±SD) of *Acanthoscelides obtectus* as a function of time and concentration of essential oils of *Echinops giganteus* (a), *Piper capense* (b) and *Xylopia parviflora* (c) on filter paper. CM: corrected mortality; SD: standard deviation



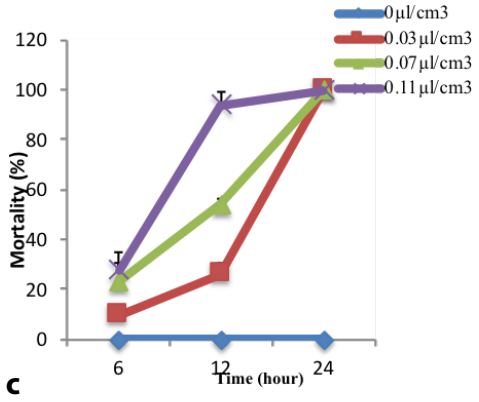




**Fig 2:** Mortality percentage (CM±SD) of *Acanthoscelides obtectus* as a function of time and concentration of essential oils of *Mondia whitei* (a), *Piper capense* (b) and *Xylopia parviflora* (c) fumigated. CM: corrected mortality; SD: standard deviation.

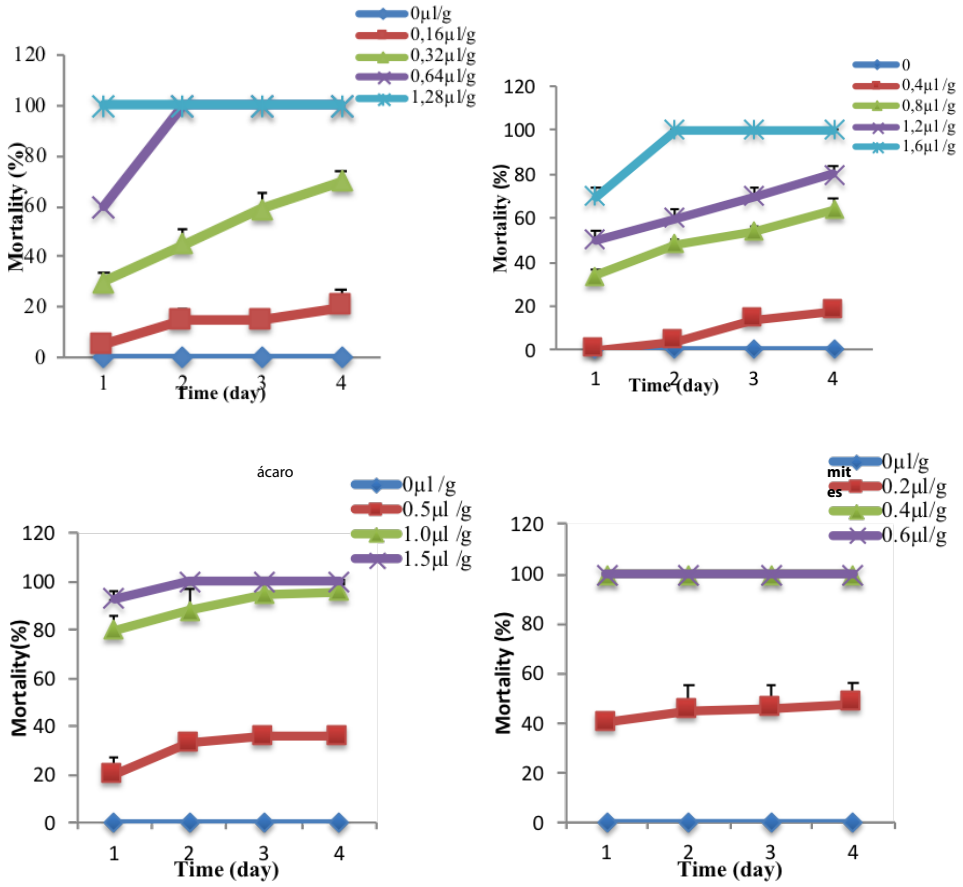



**Fig 3: M**ortality (CM±SD) of *Acanthoscelides obtectus* as a function of time and concentration of essential oils of *Echinops giganteus* (a), *Mondia whitei* (b), *Piper capense* (c) and *Xylopia parviflora* (d) on bean grains. CM: corrected mortality; SD: standard deviation

Effect of essential oils on F1 progeny production of Acanthoscelides obtectus infesting stored beans

All doses of essential oils caused significant reduction in F1 progeny of *A. obtectus* (Tab. 1). No progeny emerged in grains treated with the highest dosages of the four essential oils. Subsequently, all the doses of the oils reduced the grain weight loss, since it is proportional to the number of insects that emerge.

**Tab. 2:** Effect of dosage of essential oils of *Echinops giganteus*, *Piper capense, Mondia whitei* and *Xylopia parviflora* on F1 progeny production in stored beans and weight of beans after emergence.

| Plant | Dose (µl/g of grains) | Number of emerged insects (F1) | Percentage of inhibition of adults at F1 | Weight of bean after emergence |
|---|---|---|---|---|
| Echinops giganteus | 0.00 | 273.00±88.29a | 00.00 | 46.30±0.03a |
| | 0.16 | 65.00±6.82b | 76.19 | 48.12±0.02b |
| | 0.32 | 10.00±4.92b | 96.32 | 49.98±0.01c |
| | 0.64 | 0.00 ± 0.00c | 100.00 | 50.00 ± 0.00c |
| | 1.28 | 0.00 ± 0.00c | 100.00 | 50.00 ± 0.00c |
| Mondia whitei | 0.00 | 220.00±9.50a | 00.00 | 46.22± 0.22a |
| | 0.4 | 178.00± 4.65b | 19.09 | 47.12± 0.06b |
| | 0.8 | 112.00 ± 1.50c | 49.09 | 48.60 ± 0.12c |
| | 1.2 | 24.00± 3.36d | 89.09 | 49.86 ± 0.04d |
| | 1.6 | 0.00 ± 0.00e | 100.00 | 50.00 ± 0.00e |
| Piper capense | 0.00 | 73.00±43.85a | 0.00 | 48.33 ± 0.97a |
| | 0.5 | 43.00 ± 34.68a | 41.09 | 49.08± 0.84a |
| | 1.0 | 0.00 ± 0.00b | 100.00 | 50.00 ± 0.00b |
| | 1.5 | 0.00 ± 0.00b | 100.00 | 50.00 ± 0.00b |
| Xylopia parviflora | 0.0 | 331.00 ± 45.23a | 0.00 | 46.84 ± 0.36a |
| | 0.2 | 299.00± 84.62a | 10.21 | 46.94 ± 1.23a |
| | 0.4 | 00.00 ± 0.00b | 100.00 | 50.00 ± 0.00b |
| | 0.6 | 00.00 ± 0.00b | 100.00 | 50.00 ± 0.00b |

Anti-fungal activity of extracted essential oils

Several fungal species were isolated and identified on bean seeds collected in Dschang town; the main species were *Aspergillus flavus, Aspergillus niger, Fusarium oxysporum, penicillium sp., Rhizopus stolonifer, Rhizoctonia solani* and *Mucor* sp. The genus *Aspergillus* is most prevalent (58%) followed by the genera *Fusarium* (16%), *Penicillium* (12%), while the genera *Mucor* (6%), *Rhizopus* (3%), *Rhizoctonia* (2%) and others (3%) were the least prevalent.

*Mondia whitei* essential oil showed the best antifungal activities (MIC = 0.06 – 1.02 mg/ml). Among the eight fungal species tested, *A. niger* was the most sensitive to the treatment, while *Penicillium* sp., *F. oxysporum* and *F. moniliforme* were the most resistant. *Piper capense*, *D. glomerata* and *X. parviflora* showed low antifungal activity on all fungal isolates tested with MIC values between 4.1 and 16.32 mg/ml while *E. giganteus* did not show any antifungal activity.

**Tab. 3**: Minimal Inhibitory Concentrations (MIC) and Minimal Fungicidal Concentrations (MFC) results for antifungal activity of essential oil from *Mondia whitei.*

| | ***Mondia whitei*** | | **Mancozeb** | |
|---|---|---|---|---|
| **Fungi species** | **CMI(mg/ml)** | **CMF (mg/ml)** | **CMI(mg/ml)** | **CMF(mg/ml)** |
| *A. flavus* | 0.51 | 0.51 | 0.51 | 1 |
| *A. niger* | 0.06 | 0.51 | 0.51 | 1 |
| *F. solani* | 0.25 | 0.51 | 0.25 | 1 |
| *F. nivale* | 0.51 | 1.02 | 0.25 | 1 |
| *Penicillium sp.* | 1.02 | 2.04 | 0.25 | 1 |
| *F. oxysporum* | 1.02 | 2.04 | 0.12 | 1 |
| *F. crookwellense* | 0.51 | 2.04 | 0.12 | 1 |
| *F. moniliforme* | 1.02 | 1.02 | 0.51 | 1 |

## Conclusion

A good level of control of *A. obtectus* was achieved with essential oils of *E. giganteus*, *M. whitei*, *P. capense* and *X. parviflora*, which successfully reduced *A. obtectus* progeny production and bean loss. At the same time, the antifungal activity of these essential oils gives new opportunity for the control

of bean pathogens. Overall, essential oils extracted from these spices could play an important role in stored bean protection and reduce the risk associated with the use of synthetic insecticides.

### References

ABBOTT W. S., 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18. 265–267.

BLISS C., 1938. The determination of the dosage-mortality curve from small numbers. Q. J. Pharmacol. 11. 192–216.

CHAMPION R., 1997. Identifier les champignons transmis par les semences. Institut National de la Recherche Agronomique. Paris, France, 401p.

FINNEY D. F., 1971. Probit analysis. Cambridge University Press, Cambridge.

MARTHUR S. B., KONGSDAL O., 2003. Common Laboratory Seed Health Testing Methods for Detecting Funfi.Frederiksberg, Denmark, 425p.

NGONO N. A., BIYITIL., AMVAM Z. P.H., BOUCHET PH., 2000. Evaluation of antifungal activity of extracts of two Cameroonian Rutaceae: Zanthoxylum leprieurii Guill. Et Perr. and Zanthoxylum xanthoxyloides Waterm. Journal of Ethnopharmacology.70. 335-342

PESSOA E. B., FRANCISCO DE ASSIS C. ALMEIDA, NETO A. F., VIEIRA J. F., 2016.Treatment of bean seeds with plant extracts for controlling Zabrotes subfasciatus and its effects on physical and physiological quality during storage. African Journal of Agricultural Research.11. 4233-4241

REGNAULT-ROGER C., PHILOGÈNE B. J., VINCENT C., 2002. Biopesticides d'origines végétales. Tec et Doc-Lavoisier. Eds, Paris, p 337.

RUPOLHO G., GUTKOSKI L. C., MARTINS I. R., ELIAS M. C., 2006.Effects of grain moisture and hermetic storage on fungi contamination and mycotoxin production in oats. Ciênc.Agrotec.30. 118-125.

## Sustained effective use of phosphine in stored product protection in India: Role of UPL Limited

**Ujjwal Kumar***

UPL Limited, Mumbai, India
*Corresponding Author: ujjwal.kumar@uniphos.com
DOI 10.5073/jka.2018.463.203

Phosphine has a predominant role in stored products protection in India since more than 4 decades. Its use has gained further prominence ever since methyl bromide has been withdrawn (except QPS applications) on environmental concerns. Accordingly, the use of phosphine is being expanded to QPS treatment of certain commodities. Phosphine has several merits and as a stored product fumigant. However, there is a concern about occasional failure to achieve desired 100% mortality of insect pests during phosphine treatment in the country. Hence the factors contributing for control failures have been identified. Also there are reports about need to improve existing fumigation practices and to create awareness about the required parameters to ensure successful treatments. In this context UPL Limited, a leading manufacturer of metal phosphide formulations in the world took important steps: A. To create awareness about proper sealing of fumigation enclosures, phosphine dosage, exposure period and target terminal concentration parameters B. To impart practical demonstrations to the stakeholders in across the country details of phosphine fumigation workshops, demonstrations and industry & end user & farmers interactions conceptualized, funded and executed by UPL Limited in coordination with other lead agencies, will be discussed. Furthermore, focus on the use of on-site phosphine generators which has the advantage of rapid generation and even distribution of the gas facilitating successful treatments by way of demonstration to different end users has also been presented.

## Recent Developments in the Global Application of ECO2FUME® and VAPORPH3OS® Phosphine Fumigants

**Justin Tumambing[1]*, Courtney Christenson[2], Arda Taner[3], Dino Amoroso[4]**

[1]Solvay, Cytec Australia Holdings Pty Ltd, P.O. Box 7125, Baulkham Hills, NSW 2153, Australia
[2]Solvay Canada Inc., 9061 Garner Road, Niagara Falls, Ontario L2E 6T4, Canada
[3]Solvay USA
[4]Solvay Turkey
*Corresponding Author: J. Tumambing (Justin.Tumambing@solvay.com)