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Bioefficacy of Bersama Abyssinica Extracts against Cowpea Beetle; *Callosobruchus Maculatus* in Storage

Never Mwambela Zekeya
School of Life Science and Bio-Engineering
Nelson Mandela African Institution of Science and Technology, Arusha, Tanzania
Francis Shahada
School of Life Science and Bio-Engineering
Nelson Mandela African Institution of Science and Technology, Arusha, Tanzania
Musa Chacha
School of Life Science and Bio-Engineering
Nelson Mandela African Institution of Science and Technology, Arusha, Tanzania

Abstract:
Callosobruchus maculatus is a major pest of stored leguminous seed including black gram, green gram, cowpea and several other pulse grains. The development of eco-friendly pesticides has been a paramount in the prevention of post harvest loss. This study was therefore conducted to evaluate the efficacy of Bersama abyssinica extracts against Callosobruchus maculates (cowpea beetle). The bioassays were performed by two toxicity assays namely; extract seed dressing assay and direct toxicity by dipping where insecticidal activity was recorded by considering concentration and volume applied with mortality rate evoked. The result showed that both Bersama abyssinica methanolic and aqueous extracts caused insect mortality in a range of 60.25 ± 2.50% - 100 ± 0.00% at the rate of 0.5 - 2.0 mL/25g of cowpea seeds in direct toxicity by dipping assay. They also induced mortality ranging from 76.67 ± 3.33% to 100.00 ± 0.00% in extract seed dressing toxicity assay where extracts were tested at concentration range of 0.02-0.06 g/ml. The overall results shows that Bersama abyssinica extracts could be effective insecticidal agents against Callosobruchus maculatus (cowpea beetle) and thus improves food and nutritional security in cowpea seeds.

***Keywords**: Cowpea, Callosobruchus Maculatus, Bersama abyssinica, bioassay, food security*

1. Introduction

Cowpea is good source of dietary protein and nutritionally supplement to low-protein cereal foods that is essential for developmental processes to human (Murdock et al., 2008; Timko et al., 2007). It is also consumed by non human animals and insects as it is a vital source of protein which is then indirectly consumed by humans through the diet. Thus protection of stored pulses is of great importance to meet nutritional demand (Graham et al., 2007). Cowpea is one of the major sources of income for farmers and traders in Africa due to its ability to thrive in the semi-arid tropics on relatively poor soils and even in low rainfall. It is thus a reliable source of income and food to peasants and subsistent farmers in Africa (Roncoli et al., 2001; Singh et al., 2003). Despite the role played by cowpea in agricultural sector, post harvest infestation is a main challenge encountered toward achieving development goals as it causes massive losses in storage (Adedire *et al.*, 2011). One of the major pest of stored food is *Callosobruchus maculatus* infesting seeds by feeding on its inner protein thus causing nutrients loss for human consumption as well as seed loss for germination in agricultural activities (Epidi et al., 2008; Macedo et al., 2003). More over *Callosobruchus maculatus* infest cowpeas not only in post harvest period but also in the field thus greatly affecting food production and availability to producers and consumers (Ajayi and Lale, 2001; Baoua et al., 2012). However poor post harvest management of cereals and pulses such as favorable conditions for pest growth has been pointed to accelerate high losses in the world thus formation of unfavorable conditions by application of plant extracts inevitable to protect legumes (Boxall, 2001; Rahman and Talukder, 2006). For instance, it is estimated that maize loss due to poor post harvest management is 20 - 30% (Tefera, 2012). These cumulative post-harvest losses and quality deterioration caused by this insect are the major obstruction to achieving food security in developing countries including Tanzania (Cooper and Dobson, 2007; McBeath and McBeath, 2010; van Gogh et al., 2013). This is due to the fact that food security relies heavily on availability and safety of cereals

and pulse or locally available crops such as cowpea at household and national level thus insect infestation lead to food insecurity (Chung, 1998; Renzaho and Mellor, 2010).
The application of botanicals for pest controls of stored crops has gained popularity because are believed to be less toxic to human and environmentally acceptable (Dubey et al., 2009). The use of botanicals either alone or in synergy for control of *Callosobruchus maculatus* in both field and in stored grains has been proved to be effective and efficiency (CI, 2005; Rajapakse and Van Emden, 1997; Yule and Srinivasan, 2014). This is because natural products from plant have been proved to be environmentally friendly due to their biodegradability and affordable by peasants than synthetic pesticides which have been reported to be relatively toxic to humans and deleterious to human and animal health upon consumption or inhalation (Akhtar and Isman, 2013; Forget, 1991). Search for botanical pesticides is or paramount importance for prevention of post harvest loss (Rajapakse, 2006). It is in this vein that *Bersama abyssinica* leaves, stem bark and root bark were evaluated for their insecticidal activity against *Callosobruchus maculatus*.

2. Material and Methods

2.1. Collection of Plant Materials and Extraction
Leaves, stem bark and root bark of *Bersama abyssinica* were collected from Ilolo village of Rungwe district in Mbeya, Tanzania. Authentication was done by Ahmed Mdolwa of Tanzania Forestry Research Institute (TAFORI) and voucher specimen (BANZ 0114) was kept at Nelson Mandela African Institution of Science and Technology.

2.2. Preparation of aqueous and methanolic extracts of the tested plant parts
Leaves, stem bark and root bark of *Bersama abyssinica* were air dried under the shade and pulverized separately with an electric blender and passed through a sieve with the mesh size of 0.25 mm. Each powdered material was kept separately in an airtight container ready for soaking. Aqueous extracts were prepared by weighing 5 g of each pulverized plant part and soaked in 50 mL of hot water and left for 24 h to infuse. The filtrates were sieved through a mesh size of 0.05 mm. The filtered solution obtained was evaporated in a water bath at 60° C, then after complete evaporation, *Bersama abyssinica* leaves, stem bark and root bark extracts were weighed and preserved in air tight bottles in a refrigerator at -20° C until used for insect bioassays. Methanolic extracts were prepared by dissolving 250 g of each pulverised plant materials in 850 mL of methanol for 48h. The respective extracts were filtered through muslin cloth on a plug of glass wool in a glass column and methanol were evaporated in vacuum using a rotary evaporator and stored in refrigerator at -20°C.

2.3. Collection and Preparation of Cowpea Seeds
Cowpea seeds were obtained from a newly stocked seeds free of insecticides at Arusha market. The cowpea seeds were first cleaned and disinfested by washing in sterilized clean water then dried in air and kept in fridge for at -5^{o}C for 7 days to kill all hidden insects. The disinfected cowpeas were warmed in an oven at 40 °C for 4 h and then air dried in the laboratory to prevent moldiness before they were stored in plastic containers with perforated tight lids (Adedire et al., 2011).

2.4. Insect culture
Callosobruchus maculatus were obtained from Sokoine University of Agriculture, Morogoro-Tanzania. The insects were raised on uninfested local cultivar of cowpea and subsequent insects were drawn from this culture.

2.5. Seed Toxicity Assay
The seed toxicity assay was performed according to the method developed by Omotoso, 2008 with some modifications. Extracts were dissolved in dimethyl sulphoxide (DMSO) to make a concentration range of 0.5, 1 and 2 mg/mL which were then adsorbed on 25 g of seeds and tumbled for 5 minutes to ensure thorough mixing. The cowpea seeds were then oven-dried at 40 °C for 4 h as reported by Santhoy and Rejesus (1975). Thereafter five pairs of newly emerged adults of *C. maculatus* were introduced into the rearing boxes together with the seeds in each treatment. The set up was covered by nylon mesh and held in place with rubber bands to prevent insects from escaping. The same procedure was adopted for aqueous extracts. In the control trials, uninfested cowpea seeds were oven dried as described before and 5 pairs of newly emerged adults of *C. maculatus* were introduced and covered by nylon mesh. Each of the experiments was performed in four replicate. The experiments were monitored for 5 days and data were collected daily on insect mortality where percentage mortality was calculated using the following formula:

$$\% \text{ mortality} = \frac{\text{No of dead insects x 100}}{\text{Total number of insects}}$$

2.6. Direct toxicity by dipping method
Direct toxicity assay was performed according to the method described by Talukder and Howse, 1994 with some minor modifications. One gram (1g) *of Bersama abyssinica* leaves, stem bark and root bark extracts were separately dissolved in 1ml of dimethyl sulphoxide (DMSO) and water to make 2, 4 and 6% solutions respectively. Then five pairs of adult (2-3 days) *Callosobruchus maculatus* were placed at the center of Whatman filter paper which were then twisted to enclose the insects so as to prevent them from escaping and ensure efficiency dipping into extracts. Then the filter papers with five pairs of insects were dipped in diluted extract and

control solution for 30 seconds. Thereafter the insects were removed, air-dried and returned to Petri dishes containing 5g of cowpea seeds in which four replications were made for each treatment. Insect mortality was observed 24, 48 and 72 hours after treatment. Insects were examined daily by probing them with sharp object and those made no response were considered dead. Insect mortality data were recorded and transformed into percentage values followed by ANOVA analysis expressed as concentration per dead insects (%).

3. Results

The efficiency of *Bersama abyssinica* leaf, stem bark & root bark methanolic and aqueous extracts are shown in Table 1, 2, 3 and 4 below. The result showed that all extracts had effect on the survival of cowpea beetle, *C. maculatus* at different duration and treatments. In each treatment the mortality of cowpea beetle increased progressively with time of exposure and increase in concentration.

The *Bersama abyssinica* methanolic root bark was the most active; it evoked mortality of 77.50 ± 2.50 at 0.5 mg/mL and mortality of 100.00 ± 0.00% at concentrations of 1.0 and 2.0 mg/mL per 25g of cowpea. Stem bark methanolic extract had much higher activity compared to leaf methanolic extract which induced mortality of 75.00 ± 5.00 and 100.00 ± 0.00 % at concentration of 0.5 mg/mL and both 1.0 & 2.0 respectively. The leaf extract exhibited mortality of 60.25 ± 2.50, 97.50 ± 2.50 and 100.00 ± 0.00 % in doses of 0.5, 1.0 and 2.0 mg/mL in the extract seed dressing assay. However the methanolic extract of stem bark was very effective against *C. maculatus* by causing 100 ± 0.00 % mortality of adult *C. maculatus* at both concentrations of 0.06 g/ml and 0.04g/ml and caused 86.67 ± 3.33 % mortality at 0.02 g/ml within only 3 days whereas the study done by Ileke (2013) (Ileke et al., 2013) on the effect of plant powder by contact toxicity was reported to cause 100.00 ± 0.00 % mortality within 4 days. This activity on *C. maculatus* could be attributed by the presence of active compounds which have reported by other author(Djemgou et al., 2010; Tapondjou et al., 2006).

Aqueous extracts displayed moderate activity with similar trend of activity of the plant parts. Root bark aqueous extract induced mortality of 57.50 ± 2.50, 90.00 ± 0.00 and 100.00 ± 0.00 at concentrations of 0.5, 1.0 and 2.0 respectively. Stem bark extract at concentration of 0.5, 1.0 and 2.0 caused mortality of 40.00 ± 0.00, 72.50 ± 2.50 and 92.50 ± 2.50% respectively. Aqueous leaf extract displayed the least mortality rate of 27.50 ± 2.50% at 0.5 mg/mL but had similar activity at 1.0 mg/mL with the stem bark aqueous extract.

The result of direct toxicity by dipping depicted in Table 3 and 4 showed that methanolic extracts were more effective in inducing insect mortality as compared to aqueous extracts. The leaf methanolic extract caused mortality of 83.33 ± 3.33, 96.67 ± 3.33 and 100.00 ± 0.00% at concentrations of 0.02, 0.04 and 0.06 mg/mL respectively. The stem bark methanolic extract were effective by 86.67 ± 3.335% at 0.02 mg/mL and 100.00 ± 0.00% mortality at both 0.04 and 0.06 mg/mL. Root bark methanolic extract induced mortality of 76.67 ± 3.33, 86.67 ± 3.33 and 96.67 ± 3.33% at concentrations of 0.02, 0.04 and 0.06 respectively.

Aqueous extracts were less effective against *C. maculatus* in which there was no any plant part which exhibited 100% mortality. Even at the concentration of 0.06 mg/mL of *Bersama abyssinica* leaf, stem bark and root bark extracts the mortality recorded was 76.67 ± 3.33, 70.00 ± 0.00 and 73.33 ± 3.33% respectively. Although aqueous extracts of *Bersama abyssinica* leaves, stem bark and root bark exhibited low to moderate insecticidal activity in direct toxicity by dipping as compared to methanolic extracts still is effective as compared to other aqueous extracts of various combined plant studied (CI, 2005). This is because at the concentration of 0.06 g/ml, *Bersama abyssinica* aqueous extracts of leaves, stem bark and root bark were able to cause insect mortality by 76.67± 3.33, 70.00 ± 0.00 and 73.33 ± 3.33% in that order whereas in the control experiment the mortality was only 13.33 ± 0.00 %.This result shows that aqueous extracts of *Bersama abyssinica* are effective insecticides as compared to aqueous extracts of aromatic medicinal plants which was effective when applied in higher concentration within seven days (Omotoso, 2008).

Extracts	0.5 mg/mL	1.0 mg/mL	2.0 mg/mL
Leaf methanolic	60.25 ± 2.50	97.50 ± 2.50	100.00 ± 0.00
Stem bark methanolic	75.00 ± 5.00	100.00 ± 0.00	100.00 ± 0.00
Root bark methanolic	77.50 ± 2.50	100.00 ± 0.00	100.00 ± 0.00
Leaf aqueous	27.50 ± 2.50	72.50 ± 2.50	100.00 ± 0.00
Stem bark aqueous	40.00 ± 0.00	72.50 ± 2.50	92.50 ± 2.50
Root bark aqueous	57.50 ± 2.50	90.00 ± 0.00	100.00 ± 0.00
DMSO (control)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
H_2O	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Table 1: Callosobruchus maculatus percentage mortality exhibited by Bersama abyssinica extracts adsorbed on cowpea seeds within 5 days

Extracts	0.02 g/mL	0.04 g/mL	0.06 g/mL
Leaf methanolic	83.33± 3.33	96.67 ± 3.33	100.00 ± 0.00
Stem bark methanolic	86.67 ± 3.33	100.00 ± 0.00	100.00 ± 0.00
Root bark methanolic	76.67 ± 3.33	86.67± 3.33	96.67 ± 3.33
Leaf aqueous	30.00 ± 0.00	46.67 ± 3.33	76.67 ± 3.33
Stem bark aqueous	36.67 ± 3.33	53.33 ± 3.33	73.33 ± 3.33
Root bark aqueous	36.67 ± 3.33	53.33 ± 3.33	73.33 ± 3.33
DMSO (control)	00.0 ± 0.00	00.00 ± 0.00	00.00 ± 0.00
H_2O	00.00 ± 0.00	00.00 ± 0.00	00.00 ± 0.00

Table 2: Callosobruchus maculatus percentage mortality exhibited by Bersama abyssinica extracts in the direct toxicity by dipping assay

4. Discussion

Results reported in this study showed that *Bersama abyssinica* leaf, stem bark and root bark extracts posses insecticidal effects on *C. maculatus* commonly known as cowpea beetle. It has also been established that the solvents used for extraction plays a big role in the type of secondary metabolites extracted. Methanol seemed to extract secondary metabolites with high potency against *C. maculates*. This is in agreement with the results reported by (Ogunleye and Adefemi, 2007)on *Garcinia kolae* methanolic extract against *Callosobruchus maculates* and *Sitophilus zeamais*. The mortality exhibited ranged from 87.5% to 100% and 70% to 100% at concentrations of 0.33 g/mL and 0.20 mg/mL (Ogunleye and Adefemi, 2007). This range is in agreement with activity exhibited with *Bersama abyssinica* leaf, stem bark and root bark extracts.

Despite the fact that *B. abyssinica* is not traditionally used for the prevention of post harvest loss, current findings have demonstrated that it can be utilized by peasants in Mbeya where this plant flourishes. There are many plants in Tanzania that are traditionally used for the management of post harvest losses. For instance, (Ngowi et al., 2007) reported that botanicals are highly applied by northern Tanzanian farmers for protecting variety of plants from pest infestation. In addition to that many small-holder farmers in Africa rely on plants as source of insecticidal agents because botanical pesticides are available at low cost, user and environmentally friendly(Abate et al., 2000; Deng et al., 2009).

There are several other studies which have proven the effectiveness of plant extracts as potential insecticidal agents in controlling *C.maculatus*. For instance,(Boeke et al., 2004) reported that African plants were able to evoke toxicity and repellency activity in *C.maculatus* effectively. Another study done by (Rahman and Talukder, 2006) revealed the efficacy of plant extracts in protecting regumes through inhibiting the growth and development of cowpea weevil.

However, in the present study *Bersama abyssinica* has displayed effective insecticidal activity against *C.maculatus* as compared to the study done by Omotoso (2008) which exhibited activity at the highest concentration highlighting that it could be potential insecticide.

5. Conclusion

Therefore, these botanicals could be used as an alternative to synthetic chemicals for the control of *C.maculatus* in stored cowpea seeds. Furthermore, the local availability of these botanicals makes its access easy for small holder farmers and reduces the expenses of cowpea seed production at household and even at community level. Also these botanicals are bio-friendly and provide food safety in terms of replacing the more hazardous toxic synthetic insecticides. However this study suggests further investigation of insecticidal activities of Bersama abyssinica extracts in large scale and under pest natural environment

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7. References

1. Abate, T., Van Huis, A., Ampofo, J., 2000. Pest management strategies in traditional agriculture: an African perspective. Annual Review of Entomology. 45, 631-659.
2. Adedire, C. O., Obembe, O. M., Akinkurolere, R. O., Oduleye, S. O., 2011. Response of Callosobruchus maculatus (Coleoptera: Chrysomelidae: Bruchinae) to extracts of cashew kernels. Journal of Plant Diseases and Protection. 118, 75.
3. Ajayi, F., Lale, N., 2001. Seed coat texture, host species and time of application affect the efficacy of essential oils applied for the control of Callosobruchus maculatus (F.)(Coleoptera: Bruchidae) in stored pulses. International Journal of Pest Management. 47, 161-166.
4. Akhtar, Y., Isman, M. B., Plant natural products for pest management: the magic of mixtures. Advanced Technologies for Managing Insect Pests. Springer, 2013, pp. 231-247.
5. Baoua, I., Margam, V., Amadou, L., Murdock, L., 2012. Performance of triple bagging hermetic technology for postharvest storage of cowpea grain in Niger. Journal of Stored Products Research. 51, 81-85.
6. Boeke, S., Baumgart, I., Van Loon, J., Van Huis, A., Dicke, M., Kossou, D., 2004. Toxicity and repellence of African plants traditionally used for the protection of stored cowpea against< i> Callosobruchus maculatus</i>. Journal of Stored Products Research. 40, 423-438.

7. Boxall, R., 2001. Post-harvest losses to insects—a world overview. International Biodeterioration & Biodegradation. 48, 137-152.
8. Chung, K., 1998. The contribution of ICRISAT's mandate crops to household food security: a case study of four rural villages in the Indian semi-arid tropics. International Crops Research Institute for the Semi-Arid Tropics.
9. CI, A., 2005. BOTANICAL PESTICIDE MIXTURES FOR INSECT PEST MANAGEMENT ON COWPEA, Vigna unguiculata (L.) WALP PLANTS–2. THE POD BORER, Maruca vitrata FAB.(Lepidoptera: Pyralidae) AND POD SUCKING BUG, Clavigralla tomentosicollis STAL (Heteroptera: Coreidae). Agricultura Tropica et Subtropica. 38, 2.
10. Cooper, J., Dobson, H., 2007. The benefits of pesticides to mankind and the environment. Crop Protection. 26, 1337-1348.
11. Deng, A., Ogendo, J., Owuor, G., Bett, P., Omolo, E., Mugisha-Kamatenesi, M., Mihale, J., 2009. Factors determining the use of botanical insect pest control methods by small-holder farmers in the Lake Victoria basin, Kenya. African Journal of Environmental Science and Technology. 3, 108-115.
12. Djemgou, P. C., Hussien, T. A., Hegazy, M.-E. F., Ngandeu, F., Neguim, G., Tane, P., Mohamed, A.-E.-H. H., 2010. C-Glucoside xanthone from the stem bark extract of Bersama engleriana. Pharmacognosy research. 2, 229.
13. Dubey, N., Kumar, A., Singh, P., Shukla, R., Exploitation of natural compounds in eco-friendly management of plant pests. Recent developments in management of plant diseases. Springer, 2009, pp. 181-198.
14. Epidi, T. T., Nwani, C., Udoh, S., 2008. Efficacy of some plant species for the control of cowpea weevil (Callosobruchus maculatus) and maize weevil (Sitophilus zeamais). Int. J. Agric. Biol. 10, 588-590.
15. Forget, G., 1991. Pesticides and the third world. Journal of Toxicology and Environmental Health, Part A Current Issues. 32, 11-31.
16. Graham, R. D., Welch, R. M., Saunders, D. A., Ortiz-Monasterio, I., Bouis, H. E., Bonierbale, M., De Haan, S., Burgos, G., Thiele, G., Liria, R., 2007. Nutritious subsistence food systems. Advances in Agronomy. 92, 1-74.
17. Ileke, K. D., Bulus, D. S., Aladegoroye, A. Y., 2013. Effects of three medicinal plant products on survival, oviposition and progeny development of cowpea bruchid, Callosobruchus maculatus (Fab.)[Coleoptera: Chrysomelidae] infesting cowpea seeds in storage. Jordan Journal of Biological Science. 6, 61-66.
18. Macedo, M. L. R., Damico, D. C. S., Freire, M. d. G. M., Toyama, M. H., Marangoni, S., Novello, J. C., 2003. Purification and characterization of an N-acetylglucosamine-binding lectin from Koelreuteria paniculata seeds and its effect on the larval development of Callosobruchus maculatus (Coleoptera: Bruchidae) and Anagasta kuehniella (Lepidoptera: Pyralidae). Journal of agricultural and food chemistry. 51, 2980-2986.
19. McBeath, J. H., McBeath, J., Plant diseases, pests and food security. Environmental Change and Food Security in China. Springer, 2010, pp. 117-156.
20. Murdock, L. L., Coulibaly, O., Higgins, T., Huesing, J. E., Ishiyaku, M., Sithole-Niang, I., 2008. Cowpea. Compendium of Transgenic Crop Plants.
21. Ngowi, A., Mbise, T., Ijani, A., London, L., Ajayi, O., 2007. Smallholder vegetable farmers in Northern Tanzania: Pesticides use practices, perceptions, cost and health effects. Crop Protection. 26, 1617-1624.
22. Ogunleye, R., Adefemi, S., 2007. Evaluation of the dust and methanol extracts of Garcinia kolae for the control of Callosobruchus maculatus (F.) and Sitophilus zeamais (Mots). Journal of Zhejiang University SCIENCE B. 8, 912-916.
23. Rahman, A., Talukder, F., 2006. Bioefficacy of some plant derivatives that protect grain against the pulse beetle, Callosobruchus maculatus. Journal of Insect Science. 6.
24. Rajapakse, R., 2006. The potential of plants and plant products in stored insect pest management. J. Agric. Sci. 2, 11-21.
25. Rajapakse, R., Van Emden, H., 1997. Potential of four vegetable oils and ten botanical powders for reducing infestation of cowpeas by< i> Callosobruchus maculatus</i>,< i> C. chinesis</i> and< i> C. rhodesianus</i>. Journal of Stored Products Research. 33, 59-68.
26. Renzaho, A., Mellor, D., 2010. Food security measurement in cultural pluralism: Missing the point or conceptual misunderstanding? Nutrition. 26, 1-9.
27. Roncoli, C., Ingram, K., Kirshen, P., 2001. The costs and risks of coping with drought: livelihood impacts and farmers' responses in Burkina Faso. Climate Research. 19, 119-132.
28. SANTHOY G., REJESUS B. M., 1975.- The development rate,body weight and reproduction capacity of Sitophilus zeamais (Motsh) reared on three natural hosts.- Physiological Entomology, 2: 311-321.
29. Singh, B., Ajeigbe, H., Tarawali, S., Fernandez-Rivera, S., Abubakar, M., 2003. Improving the production and utilization of cowpea as food and fodder. Field Crops Research. 84, 169-177.
30. Tapondjou, A. L., Miyamoto, T., Lacaille-Dubois, M.-A., 2006. Glucuronide triterpene saponins from< i> Bersama engleriana</i>. Phytochemistry. 67, 2126-2132.
31. Tefera, T., 2012. Post-harvest losses in African maize in the face of increasing food shortage. Food Security. 4, 267-277.
32. Timko, M. P., Ehlers, J. D., Roberts, P. A., Cowpea. Pulses, sugar and tuber crops. Springer, 2007, pp. 49-67.
33. van Gogh, J., van der Sluis, A., Soethoudt, J., 2013. Feasibility of a network of excellence postharvest food losses.
34. Yule, S., Srinivasan, R., 2014. Combining bio-pesticides with chemical pesticides to manage legume pod borer (Maruca vitrata) on yard-long bean in Thailand. International Journal of Pest Management. 60, 67-72.