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Comparative Assessment of Insecticidal Effect of *Azadirachta indica*, *Hyptis suaveolens* and *Ocimum gratissimum* on *Sitophilus zeamais* and *Callosobruchus maculatus*

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Abstract: The powders of the leaves of *Hyptis suaveolens*, *Azadirachta indica* and *Ocimum gratissimum* were evaluated for comparative effectiveness in controlling *Sitophilus zeamais* Mots infesting stored maize grain (*Zea mays*) and *Callosobruchus maculatus* Fab infesting stored cowpea seeds (*Vigna unguiculata*). The powders were tested at 1.5, 2.5 and 3.5/80 g of the respective food materials. The parameters compared were adult mortality rate and emergence. The three plants tested showed more adult mortality and adult emergence of *C. maculatus*, where percentage adult mortality ranged from 83.3 to 93.4% for *A. indica*; 86.7 to 90.0% for *O. gratissimum*; 93.4 to 96.6% for *H. suaveolens* while *S. zeamais* recorded 13.3 to 50% for *A. indica*; 26.7 to 50% for *O. gratissimum* and 13.3 to 30% for *H. suaveolens* with the powders. The controls showed 0 to 33.4% mortality for *C. maculatus* compared with 0 to 3.3% obtained in *S. zeamais* within the same period. After 42 days post treatment adult emergence indicated that more *C. maculatus* ranging from mean number of 13 to 68 emerged while *S. zeamais* recorded 11 to 37 within the same period of consideration. The statistical analysis indicated that where significant differences occurred, it was caused by plant type other than concentration. The performance of individual plant type revealed that *O. gratissimum* and *A. indica* were better against *S. zeamais* than *H. suaveolens* where they caused mortality ranging from 13.3 to 50% with *H. suaveolens* recording 13.3 to 30.0%. However, the mortality ability of *H. suaveolens* against *C. maculatus* was better with a mean percentage mortality range of 93.3 to 96.6% compared with 83.3 to 90.0% caused by *O. gratissimum* and *A. indica*. The general emergence trend also indicated that *H. suaveolens* performed better in reducing emergence of new adults. These suggest that botanical insecticides are promising and their effectiveness varies with species.

Key words: Mortality rate, emergence, botanical insecticide, *Sitophilus zeamais*, *Callosobruchus maculatus*.

INTRODUCTION

The utilization of plant materials to protect field crops and stored commodities against insect attack has a long history. Opinion now favours a shift away from the reliance on conventional insecticides towards the use of more natural, sustainable methods of protecting crops from insect damage (Sallam, 1999). According to KeAOEta *et al.* (2000) synthetic insecticides are expensive for subsistence farmers and they may pose potential risks owing to the lack of adequate technical knowledge related to their safe use. One alternative to synthetic insecticides is insecticidal plants; African farmers are traditionally familiar with them Thiam and Ducummon (1993). The tropical flora is a major source of plant-based insecticides Aranson *et al.* (1989). Aromatic species, particularly those in the family Labiate (or Lamiaceae) are among the most widely used plants in insect pest control Lambert *et al.* (1985), Shaaya *et al.* (1997) and Mortan (1981).

The toxicity of botanical insecticides is traceable to the presence of secondary compounds (metabolites) present in the plants. The presence of secondary compounds, which have no known function in photosynthesis, growth or other aspects of plant physiology, give plant materials or their extracts their anti-insect activity (Sallam, 1999).

The test organisms in this work are the maize weevil, (*Sitophilus zeamais* Motsch.), a curculionid and the cowpea weevil (*Callosobruchus maculatus* F.), a bruchid. The maize weevil is a field to store pest (i.e., primary pest) which attack stored maize leaving circular holes on the surface of the grain and make tunnels below the seed coat Macdonald (1989). Kossou and Bosque-perez (1995) stated that maize weevils are distinguishable from all other common storage pest by their long beak or rostrum. These are found in all warm and tropical parts of the world and have been found as causing heavy loses to farmers every year.

The cowpea weevil infests both pods in the field and seeds in storage Stoll (1988). According to Singh *et al.* (1978), 100% of cowpea seeds are infested after 3±5 months of storage in West Africa. Egbuchua (1980) and Tanzubil (1991) found that this insect can damage 100% of stored seeds of cowpea causing weight losses of up to 60%. After six months of storage, losses in terms of perforated seeds can reach 90% (Seck *et al.*, 1991). Annual losses of cowpeas in Nigeria due to *C. maculatus* have been estimated at 2900 tons (dry weight) Caswell (1973).

In Nigeria, very little research has been done on plant insecticides even though the potential appears quite high Olaifa and Akingbohungho (1986). Hence the comparative study of *Azadirachta indica*, *Hyptis suaveolens* and *Ocimum gratissimum*. In recent years, several reviews have been published which outline the use of the neem tree, *Azadirachta indica* (Meliaceae) as a botanical insecticide (Jacobson, 1989); Koul *et al.*, 1990; Schmutterer, 1990; Ascher, 1993). The mode of action of azadirachtin, the principal insecticidal constituent of neem oil has only recently been elucidated (Mordue and Blackwell, 1993).

Literatures of plant materials in dusts or extracted oils against *S. zeamais* and *C. maculatus* now abound and the need to elucidate the differences in behaviour of these two storage insect pests have necessitated this comparative bioassay work. This is against the backdrop that *S. zeamais* feeds at adult stage and the activities of the other stages occur within the grain while *C. maculatus* feeds only at its larval stage and oviposition is external i.e., on the grain surface.

MATERIALS AND METHODS

The experiment was conducted at the Postgraduate Research Laboratory of the Department of Animal and Environmental Biology of University of Benin, Nigeria.

Collection and culturing of adult insects used: Adults *S. zeamais* and *C. maculatus* were collected and cultured from infested stored maize and cowpea obtained from New Benin Market in the Benin metropolis. The culturing was done on whole grains of maize for *S. zeamais* and cowpea for *C. maculatus* at ambient temperature of 28±2°C and relative humidity ranging from 70-80%. Freshly emerged adults of *C. maculatus* and *S. zeamais* from the laboratory cultures were taken out and used for the experiment.

Plant materials and grains used: The plant materials accessed for insecticidal activity, the parts used and other relevant information are provided in Table 1. The fresh leaves of *A. indica* and *O. gratissimum* were

collected from Benin City. Fresh leaves of *H. suaveolens* were obtained from cultivation of seeds collected from Kogi State, Nigeria. Matured leaves of plant species were sun-dried for three days and ground into powder in the laboratory using a domestic electric blender. The ground forms were then weighed into concentrations of 1.5, 2.5 and 3.5g and replicated thrice per plant type and per concentration using a digital weigh balance. These were dried and stored in cool dried place and were used for the admixture experiment.

Fifty gram each of the sun-dried leaves of each plant type were extracted using 1000 mL of 95% ethanol solution by soxhlet extraction method in the chemical laboratory of Rubber Institute of Nigeria, Iyanomo. The whole extracts obtained were stored in the refrigerator for the experiment.

Methods of treatment: Each ground plant material was thoroughly admixed with 80 g disinfested food materials (maize grain) and (beans seeds) contained in separate plastic dishes with lid, at a rate of 1.5, 2.5 and 3.5 g plant material. Each concentration was replicated thrice. Ten newly emerged maize weevils were introduced into each set up containing maize grains while ten newly emerged beans weevil were introduced into each cowpea set up. The control set-ups which were void of the plant materials also had ten newly emerged adults introduced. The whole set ups were kept in the culture at room temperature of 28°C, throughout the experimental period.

The whole extract treatment was carried out with 25 g disinfested food materials treated with the 2 mL absolute extract obtained. Ten newly emerged adults were used in each case.

Periods of observation: Adult mortality was recorded at 24 h, 2, 3 and 7 days post treatment according to the duration in FAO bulletin FAO (1999), for all plant types at the different concentrations. Adult emergence was recorded at 24, 27, 30, 33, 36, 39 and 42 days post treatments.

RESULTS

The results obtained in this study indicated that *Callosobruchus maculatus* were more susceptible at all concentrations of the plant species investigated compared to *Sitophilus zeamais*. Table 1 shows the plant types and the mean mortality of insects caused by the different plant species at various concentrations. It was observed that increase in the concentrations of plant materials did not increase the mean mortality effects against *C. maculatus* as compared with *S. zeamais* were increase in concentrations resulted in an appreciable increase in mean mortality. Performance of plant type per concentration of each insect type is as indicated:

Table 1: Plant types/concentrations and mean mortality of insects at different concentrations

Insect type	<i>Hyptis suaveolens</i>			<i>Ocimum gratissimum</i>			<i>Azadirachta indica</i>			Control		
	1.5 g	2.5 g	3.5 g	1.5 g	2.5 g	3.5 g	1.5 g	2.5 g	3.5 g	1.5 g	2.5 g	3.5 g
<i>Sitophilus zeamais</i>	0.67±0.94 (0-2)	0.75±0.96 (0-2)	0.33±0.47 (0-1)	0.67±0.82 (0-1.67)	1.25±0.50 (1-2)	1.17±1.17 (0-2.33)	0.33±0.47 (0-1)	1.25±1.45 (0-2.67)	1.17±1.00 (0-2)	0.08±0.17 (0-0.33)	0.08±0.17 (0-0.33)	0.08±0.17 (0-0.33)
<i>Callosobruchus maculatus</i>	2.5±1.47 (1-4.33)	2.33±1.18 (1-3.33)	2.41±1.20 (0.67-3.33)	2.18±0.58 (1.33-2.67)	2.17±1.48 (0.33-3.67)	2.25±1.45 (1-4.33)	2.34±1.12 (0.67-3)	2.33±1.59 (0.67-4)	2.34±1.41 (0.67-3.67)	0.84±0.96 (0-1.67)	0.84±0.96 (0-1.67)	0.84±0.96 (0-1.67)

Table 2: Mean percentage mortality of *S. zeamais* and *C. maculatus* compared at different concentrations

Plant type	1.5 g plant type		2.5 g plant type		3.5 g plant type	
	<i>C. maculatus</i>	<i>S. zeamais</i>	<i>C. maculatus</i>	<i>S. zeamais</i>	<i>C. maculatus</i>	<i>S. zeamais</i>
<i>O. gratissimum</i>	90.0%	26.7%	86.7%	50%	90.0%	46.6%
<i>A. indica</i>	93.4%	13.3%	83.4%	50%	83.3%	46.7%
<i>H. suaveolens</i>	94.3%	26.7%	93.4%	30%	96.6%	13.3%
Control	33.4%	3.3%	33.4%	3.3%	33.4%	3.3%

Table 3: Plant types/periodic emergence at different concentration of plant powders

Insect type	<i>Hyptis suaveolens</i>			<i>Ocimum gratissimum</i>			<i>Azadirachta indica</i>			Control		
	1.5 g	2.5 g	3.5 g	1.5 g	2.5 g	3.5 g	1.5 g	2.5 g	3.5 g	1.5 g	2.5 g	3.5 g
<i>Sitophilus zeamais</i>	2.19±4.05 (0-11)	2.62±4.42 (0.12.33)	3.76±4.28 (0-10)	4.10±5.91 (0-15.67)	1.57±2.81 (0-7.67)	3.76±4.26 (0-10)	5.33±7.03 (0-18.33)	1.62±2.74 (0-6.67)	2.95±5.13 (0-13.33)	10.38±11.4 (1-30.67)	10.38±11.4 (1-30.67)	10.38±11.4 (1-30.67)
<i>Callosobruchus maculatus</i>	1.8±1.62 (0-4.67)	2.57±3.26 (0-9)	3.90±3.56 (0-10.33)	8.09±6.82 (1.67-21)	9.62±7.71 (1.67-23.33)	8.62±8.21 (2.5.33)	7.43±6.94 (1.67-21.33)	7.48±8.16 (1-25)	8.72±8.82 (1.67-26.67)	12.14±8.82 (0-27)	12.14±8.82 (0-27)	12.14±8.82 (0-27)

Range in parenthesis

At 1.5 g: *Callosobruchus maculatus*: *Hyptis suaveolens* > *Azadirachta indica* > *Ocimum gratissimum* > Control
Sitophilus zeamais: *H. suaveolens* = *O. gratissimum* > *A. indica* > Control
 At 2.5 g: *Callosobruchus maculatus*: *H. suaveolens* > *O. gratissimum* > *A. indica* > Control
Sitophilus zeamais: *O. gratissimum* = *A. indica* > *H. suaveolens* > Control
 At 3.5 g: *Callosobruchus maculatus*: *H. suaveolens* > *O. gratissimum* > *A. indica* > Control
Sitophilus zeamais: *A. indica* ≥ *O. gratissimum* > *H. suaveolens* > Control

The result also indicated that *Hyptis suaveolens* effected greater insecticidal action against *Callosobruchus maculatus* across the concentrations from 1.5 to 3.5 g plant powders compared to *Ocimum gratissimum* and *Azadirachta indica*. At higher concentrations of 2.5 and 3.5 g plant powders, *Ocimum gratissimum* and *Azadirachta indica* showed better morality effect against *Sitophilus zeamais* than *H. suaveolens* except at 1.5 g concentration where *H. suaveolens* equals average death caused by *O. gratissimum* as indicated in Table 2.

Table 3 shows plant types and mean emergence at different concentrations of plant powders. The trend of periodic emergence indicates that *Hyptis suaveolens* was better in preventing the emergence of both *Callosobruchus maculatus* and *Sitophilus zeamais* compared to *Ocimum gratissimum* and *Azadirachta indica* and Control.

Summarily, across concentrations and plant types; more emergences were obtained in the trend indicated below:

Callosobruchus maculatus: Control > *Ocimum gratissimum* > *Azadirachta indica* > *Hyptis suaveolens*
Sitophilus zeamais: Control > *O. gratissimum* > *A. indica* > *H. suaveolens*

However, *Ocimum gratissimum* and *Azadirachta indica* were more concentration dependent in causing the periodic emergence of *Sitophilus zeamais*. Less number of *S. zeamais* emerged at higher concentrations of 2.5 and 3.5 g. The emergence of *Callosobruchus maculatus* was independent of concentration in the plant types under consideration.

DISCUSSION

The use of indigenous plant products and other locally available materials to protect stored cereals and legumes have been reported by many workers (Golob *et al.*, 1982; Stoll, 1992; Lale, 1995).

Golob and Webely (1980) had advanced that oils are particularly more effective against bruchids which lay their eggs outside the grain and less effective against curculionids which insect their eggs inside the grains. This present finding revealed that the use of plant powders followed a similar pattern. *Callosobruchus maculatus* a bruchid showed a general higher mortality at all considered concentrations compared to *Sitophilus zeamais* a curculionid. The work revealed that the mortality pattern of *C. maculatus* to the powders were

independent of concentrations whereas mortality of *S. zeamais* increased with increase in concentrations. This is probably due to difference in the behavioural tendency of both insects. *S. zeamais* feeds and bores into grains thus making them to require more dosages for kill whereas, *C. maculatus* are more exposed to the effects of powders as adult spend more time in contact with the plant powders which justifies their mortality being independent of concentrations.

The comparative effectiveness of each of the plant types against the two weevils revealed that the plant type were generally more effective against *C. maculatus* than *S. zeamais* and the mortality effect of each plant type on the weevil were a characteristic inherent in the plants. The higher mortality on *C. maculatus* than *S. zeamais* could probably be attributed to the fact that, the adults are short-lived while the latter feeds at all stages of its life cycle and inserts its eggs inside the grains. This would mean requiring more dosage of plant powders, extracts or oils that would be lethal to them as indicated by the trend of the mortality shown in the result.

The trend of mortality shown was also dependent on plant type. *A. indica* and *O. gratissimum* showed greater insecticidal effect against *C. maculatus* and *S. zeamais*. The mortality effect of *H. suaveolens* against *S. zeamais* decreased with concentration and was generally lower compared with *A. indica* and *O. gratissimum*. This agreed with what Lajide *et al.* (1998) reported that *H. suaveolens* has moderate insecticidal activities against *S. zeamais*.

The present study showed that the leaves of *A. indica* in powder and extract forms effected the mortality of adult *S. zeamais*. Its performance was however better with *C. maculatus* as more adults *C. maculatus* died compared to *S. zeamais*. *Ocimum gratissimum* on the other hand caused a considerable death of both *C. maculatus* and *S. zeamais*. The result obtained corroborate with the findings of Bekele and Bekele (1996), where the toxicity of *O. suare* to *S. zeamais* was reported.

The mortality caused by the different plants as already indicated could be attributed to several mechanisms. The use of plant powders could have resulted to death as a result of physical barriers effect of the plant materials. This is because the powder has the tendency of blocking the spiracles of insects thus impairing respiration leading to the death of insects. While feeding on whole grains by the weevil (*S. zeamais*) or the larvae of *C. maculatus* might pick up a lethal dose of the treatment thus resulting in stomach poisoning.

Sowumi and Akinnusi (1983) had reported that the ability of *A. indica* to control *C. maculatus* are attributable to the protectant effect of the presence of azadirachtin. Schmutterer (1990) stated that azadirachtin

has deterrent, antifeedant, growth disrupting, anti-ovipositional and fecundity reducing properties on a range of insects. Endersby and Morgan (1991) stated that *A. indica* derivations are most effective as feeding poisons for nymphs or larvae of phytophagous insect and lepidopterous larval are very susceptible. The mortality caused by *A. indica* as reported in the result is probably due to the above inherent properties.

Peerzada (1997) revealed 32 chemical components of *H. suaveolens* with 1,8-Cineole (32%) and Caryophyllene (29%) being the main constituents. Aggarwal *et al.* (2001) had reported the toxicity of 1,8-Cineole towards three species of stored product Coleopterans of which *C. maculatus* was one. The effectiveness of *H. suaveolens* are probably attributable to these or other secondary metabolites inherent in *H. suaveolens* which may have caused the death of the adult weevils by contact poisoning. It has been reported to have good grain protectant effect against *C. maculatus* for long term storage of cowpea seed, (Fatope *et al.*, 1995).

From the results obtained, despite the mortality recorded, new adult insects considered emerged and the number of emergence was a characteristic of each plant type. It is noted that though the least general average adult mortality was recorded with the *H. suaveolens* treatment, it recorded the lowest average adult emergence. These suggest that *H. suaveolens* exerted better larvicidal and ovicidal effect and hence reduced adult emergence. The result also showed that in spite of high mortality recorded in *A. indica* and *O. gratissimum*, emergence of adults *Callosobruchus maculatus* and *Sitophilus zeamais* were relatively high compared with the controls, indicating that reproduction and development were not impaired, though Schmutterer, (1990) stated that anti-ovipositional and fecundity reducing properties on a range of insects are characteristics of azadirachtin.

The result of this present study gives a clear-cut base for comparing both insects as many researchers have always investigated them individually. It is also an added information to the information data base of stored product pests.

Despite the success recorded in this investigation, it is expected that more researchers would investigate them together in-order for a clear-cut distinction to be made between the insects physiologically and behaviorally.

REFERENCES

- Aggarwal, K.K., K.A. Tripathi, V. Prajapati and K. Sushil, 2001. Toxicity of 1,8- cineole towards three species of stored product coleopterans. Insect Sci. Appl., 21: 155 -160.

- Arnason, J.T., B.J.R. Philogeane and P. Morand, 1989. Insecticides of Plant Origin. ACS Symposium Series 387, Washington, DC.
- Ascher, K.R.S., 1993. Non conventional insecticidal effects of pesticide available from the Neem tree, *Azadirachta indica* Arch. Insect Biochem. Physiol., 22: 433- 449.
- Bekele, F. and J. Bekele, 1996. Sampling of the phenetic diversity of cacao in the international Cocoa Gene Bank of Trinidad. Crop Sci., 36: 57-64.
- Caswell, G.H., 1973. The impact of infestation on commodities. Trop. Stored Prod. Infest., 25: 19.
- Egbuchua, B.N., 1980. The Biology of *Callosobruchus maculatus* (Fabs) (Coleoptera: Bruchidae) A stored product pest of cowpeas in Nigeria. Ph.D Thesis, pp: 406.
- Endersby, N.M. and W.C. Morgan, 1991. Alternatives to synthetic chemical insecticides for use in crucifer Crops. Biol. Agric. Hort., 8: 33-52.
- FAO, 1999. The use of spices and medicinal as bioactive protectants for grains. www.fao.org/docrep/x2230e/x2230e05.htm.
- Fatope, M.O., A.M. Nuhu, A. Mann and Y. Takeda, 1995. Chemical composition of the essential oil of *Hyptis suaveolens*. Molecules, 2: 165-168.
- Golob, P. and D.T. Webley, 1980. The use of plant and minerals as traditional protectants of stored products. Publication No. G138, Tropical Product Institute, UK.
- Golob, P., J. Mwanbula, V. Mlango and F. Ngulube, 1982. The use of locally available materials as protectants of maize grains against insect infestation during storage in Malawi. J. Stored Prod. Res., 18: 67-78.
- Jacobson, M., 1989. 1988 Focus on Phyto chemical Pesticides, Volume 1: The NEEM tree. CRC Press, Boca Raton, FL., pp: 178.
- KeÄÖeta, S.M., C. Vincent, J. Schonit, S. Ramaswamy and A. BeÄlanger, 2000. Effect of various essential oils on *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). J. Stored Prod. Res., 36: 355-361.
- Kossou, D.K., J.H. Mareck and N.A. Bosque-Perez, 1993. Comparism of improved and local maize varieties in the Republic of Benin with emphasis on susceptibility to *S. zeamais* Mots. J. Stored Prod. Res., 29: 333-343.
- Koul, O., M.B. Isman and C.M. Ketkar, 1990. Properties and uses of Neem, *Azadirachta indica*. Can. J. Bot., 68: 1- 11.
- Lajide, L., C.O. Adedire, W.A. Muse and S.O. Agele, 1998. Insecticidal activity of powders of some Nigerian plants against the maize weevil (*Sitophilus zeamais* Motsch). ESN Occasional Publication, 31: 227-235.
- Lale, N.E.S., 1995. An overview of the use of plant products in the management of stored product Coleoptera in the tropics. Postharvest News and Information, 6: 69N-75N.
- Lambert, J., J.T. Arnason and B.J.R. Philoge Äne, 1985. Bruchid control with traditionally used insecticidal plats *Hyptis spiceigera* and *Cassia nigricans*. Insect Science and its Application, 6: 167-170.
- Macdonald, D.W., 1984. A questionnaire survey of farmers opinions and actions towards wildlife on farmlands. ITE Symposium No. 13: Agriculture and Environment.
- Mordue, A.J. and A. Blackwell, 1993. Azadirachtin: An update. J. Insect Phys., 39: 903-924.
- Morton, J.F., 1981. Atlas of medicinal plants of Middle America. Thomas, Spring field, 11: 761-763.
- Olaifa, J.I. and A.E. Akingbohunge, 1986. Antifeedant and insecticidal effects of the passive extracts of *A. indica*, *P. guineense* on variegated grasshopper *Zonocerus variagatus* Proceeding of 3rd International Neem Conference Nairobi Kenya.
- Peerzada, N., 1997. Chemical composition of the essential oil of *Hyptis suaveolens*. Molecules, 2: 165-168.
- Sallam, M.N., 1999. Chapter 11 Insect Damage: Damage on Post-harvest. http://www.fao.org/inphol.
- Schmutterer, H., 1990. Properties and potential of natural pesticides from the neem tree, *Azadirachta indica*. Ann. Rev. Entomol., 35: 271-277.
- Seck, D., A.B. Sidibe, E. Haubruge, J.L. Hemptinne and C. Gaspar, 1991. The chemical protection of stocks of wheat and maize in Senegal. Medelingen Van de Faculteit landbouwwetenschappen Rijksuniversiteit Gen, 56/3b: 1225-1234.
- Shaaya, E., M. Kastjukorski, J. Eilberg and C. Sukprakarn, 1997. Plant oils as fumigants and contact insecticides for the control of stored product insects. J. Stored Prod. Res., 33: 7-15.
- Singh, S.R., R.A. Luse, K. Leuschner and D. Nangju, 1978. Groundnut oil treatment for the control of *Callosobruchus maculatus* (F.) during cowpea storage. J. Stored Prod. Res., 14: 77-80.
- Sowunmi, O. and O.A. Akinnusi, 1983. Studies on the use of neem kernel in the control of stored cowpea beetle, *Callosobruchus maculatus* (F.). J. Stored Prod. Res., 27: 28-31.
- Stoll, G., 1988. Protection of Natural Vegetation in Tropical Zones. CTA; AGRECOL, pp: 177.
- Tanzubil, P.B., 1991. Control of some insect pests of cowpea (*Vigna anguiculata*) with neem (*Azadirachta indica*) in Northern Ghana. Trop. Pest Manag., 37: 216-217.
- Thiam, A. and G. Ducommun, 1993. Protection of natural vegetation in Africa. Research Study Series No. 154-156. Enda-Editions, Dakar, Senegal.