

Full Length Research Paper

# Storage of open-pollinated maize variety seeds in active cattle kraal to suppress maize weevils and improve seed quality and seedling vigour

Phatu W. Mashela<sup>1\*</sup> and Kgabo M. Pofu<sup>2</sup>

<sup>1</sup>School of Agricultural and Environmental Sciences, University of Limpopo, Private Bag X1106, Sovenga 0727, South Africa.

<sup>2</sup>Agricultural Research Council – VOPI, Private Bag X293, Pretoria, 0001, South Africa.

Accepted 12 March, 2013

Open-pollinated maize (*Zea mays*) varieties remain popular in marginal communities since seeds can be selected and stored for the next season. However, maize weevil (*Sitophilus zeamais*) is an economic post-harvest pest of maize grains, which is difficult to manage. Various indigenous seed storage technologies had been in use within marginal communities, with little empirically-based support of their efficacy on weevils. The influence of storing maize seeds in active cattle kraal (ACK) for suppression of weevils and therefore protection of seeds was studied at three locations over two seasons. Water-tight plastic containers, each with 500 maize seeds infested with 20 maize weevils, with 10 replications, were stored in 30-cm-deep holes in ACK, with controls stored at farmer level. At 120 days after storage, relative to farmer controls, ACK reduced final *S. zeamais* numbers (73 to 95%) and damage of various seed sets (27 to 97%), but improved seed quality (101 to 3500%), seedling emergence (27 to 64%) and root/shoot ratio (19 to 50%). In conclusion, results of the study suggested that ACK storage has the potential for storing open-pollinated maize seeds throughout winter for farmers in marginal communities to allow for quality seeds that produce vigorous seedlings in the next growing season.

**Key words:** Active cattle kraal, maize weevil, *Sitophilus zeamais*, seed quality, seedling vigour.

## INTRODUCTION

Maize (*Zea mays*) in maize-consuming countries has since become a cash crop – with prices fluctuating in response to demand and supply economic principles (Anon, 2011). Consequently, the market-driven production inputs for maize are increasingly limiting the production of this crop in marginal communities, with the availability of high quality seeds being one of the most daunting tasks in the production chain. Commercially available maize seeds are mostly in the form of hybrids, which makes business sense since seeds have to be

purchased annually at ever-inflated prices. In support of this business sense, although hybrid seeds are chemically-treated, they store poorly at the farmer level since pesticides have short-life spans and cannot protect seeds beyond one season, particularly under farmer level conditions in tropical areas. Maize seed storage at the farmer level, particularly in tropical regions (Danho et al., 2002), has scantily been successful due to enormous losses incurred from maize weevil (*Sitophilus zeamais*) damage.

\*Corresponding author. E-mail: phatu.mashela@ul.ac.za. Tel: +27152682190. Fax: +27152682892.

Generally, *S. zeamais* female bores a hole through the seed testa, into which one oval white egg is laid (Danho et al., 2002). After withdrawing the ovipositor, the opening to the egg is plugged with a waxy secretion, which hardens and leaves a small raised area on the surface of the grain, which limits contact with pesticides. Upon egress inside the protected area, the white legless grub remains in the grain and copiously feeds on the endosperm, pupates and emerges as a young beetle, which bores an exit hole and exits as an adult. Biological duties of an adult female include mating, finding an undamaged seed, boring an entry hole, laying an egg and plugging. A female singly lays 300 to 400 eggs during its 5 to 8-month lifespan, with the egg-to-adult life cycle averaging 36 days (Danho et al., 2002). Since attack starts when cobs are still on the plants (Danho et al., 2002), eggs, grubs and/or young adults inside grains serve as a source of inoculum during storage. The endosperm is essential for successful germination and subsequent emergence of seedlings, with complete consumption of this entity resulting in total failure of germination (Campbell, 1990). Attempts to breed for resistance against this pest in seeds are underway (Siwale et al., 2009).

In Limpopo Province, South Africa, the government, through the Public-Private Partnership strategy, initiated a program where selected farmers were intensively trained to produce open-pollinated varieties (OPVs) for seed in 2000 (Anon., 2011). Cooperative seed processor and cold storage facilities were also erected. However, during the awareness campaigns, one advanced reason for choosing OPVs as opposed to hybrids was that the former provides the flexibility for buying once and then storing seeds after harvest for the next season. The unintended result had been that at harvest maize producers selected and stored their own seeds using various indigenous technologies (Anon., 2011), resulting in the hand-picked seed-producers suffering economic losses.

Generally, in various maize-producing countries, but specifically in Africa, indigenous seed storage to control *S. zeamais* has been practiced at the farmer level, particularly in marginal communities (Pierrard, 1986; Saayman, 1995). Even with the advent of synthetic pesticides, for various reasons, most endemic farmers hardly adopted advanced seed protection technologies for various reasons (Ngobeni, 2004; Ngobeni and Mashela, 2005; Saayman, 1995). For instance, use of synthetic pesticides for seed protection did not provide the much desired flexibility for consuming stored seeds should unexpected circumstances so dictate (Ngobeni and Mashela, 2005). However, results of indigenous seed storage vary from region to region, probably due to climatic variations (Ngobeni, 2004).

In southern Africa, particularly among the Sotho-speaking communities, the most preferred indigenous seed storage had been the use of an active cattle kraal

(ACK), where a hole was dug in the center of the kraal, with seeds being poured into a waterproof container - sometimes as big as a ton (Ngobeni, 2004). An ACK was traditionally a secure wooden enclosure from where cattle were taken out in the morning for grazing in the veld during the day and returned in the evening for safe-keeping throughout the night. Due to stock theft, this tradition prevails to date. Generally, ACK and caves were meant for long-term storage of large quantities of grain for consumption, particularly at the peak of wars for natural resources, while other storage types within the homestead were meant for storing small quantities. However, the influence of ACK storage on *S. zeamais* numbers, seed quality and seedling vigour for planting in the following season is not documented. The objective of our study was to investigate the influence of ACK storage at three locations with different climates on suppression of *S. zeamais* numbers and the subsequent quality of seeds and seedling vigour.

## MATERIALS AND METHODS

### Location and planting

The study was initiated in 2010 and repeated in 2011 at three provinces in South Africa: (1) Kekana Village in North West Province (25°24'27"S, 28°17'8"E) - situated in the high-veld region of South Africa with cold winters and occasional frost, (2) Moletlane Village in Limpopo Province (24°21'0"S, 29°20'0"E) - in the mid-veld region, with moderately cold winters without frost, and (3) Shatale Village in Mpumalanga Province (24°50'0"S, 31°40'E) - in the low-veld region with warm winters without frost. *Sitophilus zeamais* populations were raised on maize seeds using 500 ml plastic containers with lids at the University of Limpopo (23°53'10"S, 29°44'15"E) in a growth chamber (25°C, 40% RH). At the beginning of the planting seasons in 2010 and 2011, ZM 421 (OPV) was planted on 0.25 ha at each location in order to conduct the trials with locally-adapted seeds. At the end of the season, cobs were collected and hung unshelled in shade at the farmer's homestead to minimise infection by weevils.

### Experimental design

Team members were dispatched to each location so that the trials started on the same date after harvest. Five hundred seeds each in 500-ml plastic bottles were infested with 10 female and 10 male weevils. Ten 30-cm-deep holes were dug in the centre of ACK using a soil auger within a 2-m radius from the centre of the kraal. Ten numbered bottles were closed with their respective lids, capped halfway with a plastic bag to ensure that rainwater or urine did not come into contact with the lids and therefore, into the containers. The lids were not airtight to allow for gaseous exchange. Containers were arranged in a completely randomised design (CRD) and inserted into separate holes in an upright position. Other 10 bottles, each with 500 seeds and 20 weevils served as farmer controls at room temperature within the homestead of the ACK-participating farmer.

### Data collection

At 120 days after storage, containers were dug out on the same

**Table 1.** Mean±SE final population densities of weevils from 500 maize seeds infested with 20 weevils and stored at the farmer level and under active cattle kraal conditions at three locations in South Africa at 120 days after storage.

Treatment	First season, 2010			Second season, 2011		
	Kekana	Moletlane	Shatale	Kekana	Moletlane	Shatale
Farmer control	75 ± 4.7	44 ± 2.9	54 ± 3.1	56 ± 2.7	59 ± 3.5	37 ± 2.3
Active kraal	9 ± 1.8	8 ± 0.8	8 ± 2.2	3 ± 0.8	16 ± 1.8	5 ± 1.7
Impact (%) <sup>y,x</sup>	-88 <sup>*</sup>	-82 <sup>*</sup>	-85 <sup>*</sup>	-95 <sup>*</sup>	-73 <sup>*</sup>	-86 <sup>*</sup>

<sup>y</sup>Impact (%) = [active-kraal/farmer-control] - 1 × 100. <sup>x</sup>Impact values with <sup>ns</sup> and <sup>\*</sup> indicated that treatment means were not significant at P ≤ 0.05 and significant at P ≤ 0.05 according to two-sample Student t-test.

day and along with control containers brought to the VLIR Nematology Laboratory, University of Limpopo (23°53'10"S, 29°44'15"E) for assessment. The floating test was used to evaluate the presence and absence of embryos in seeds (Hartmann et al., 1988). Briefly, seeds were poured into a 5-L bucket, half-filled with water and seeds were classified into four: (1) floating embryo-less seeds with holes, (2) floating embryo-less seeds without holes, (3) sunken embryo-containing seeds with holes and (4) sunken embryo-containing seeds without holes. Additionally, the floating test allowed for the capturing and counting of weevils.

Five sunken embryo-containing seeds without holes/location/treatment were randomly selected, bulked and mixed – to constitute 50 seeds. These 50 seeds/treatment/location were sown in 200-hole polystyrene-seedling tray containing steam-pasteurised (300°C for 1 h) river sand under greenhouse conditions (mean day/night temperature 25/13°C) at the University of Limpopo, South Africa. A 10-hole row constituted a plot, with farmer-control and ACK-stored seeds randomly arranged in CRD, with 5 replications. Plots of the three location-based trials were irrigated to field capacity every second day and fertilised weekly with half-strength Hoagland solution (Hoagland and Arnon, 1950). At 30 days after sowing, emerged seedlings were each separated from roots and oven-dried at 68°C for 72 h for determination of dry mass.

#### Data analysis

Discrete data were transformed through  $\log_{10}(x + 1)$  to homogenise the variances (Gomez and Gomez, 1984) prior to analysis of variance through the SAS software (SAS Institute, 2004). The interactions between 2010 and 2011 seasons for the variables and locations were not significant (P ≤ 0.05). Consequently, data were re-analysed per season and per location for the two treatments (Gomez and Gomez, 1984) using two-sample Student-t test. Relative percentage impacts were computed [Impact (%) = (ACK/Control - 1) × 100] in order to establish the magnitude and direction of the impacts. Unless otherwise stated, only treatment effects significant at the probability level of 5% were discussed.

## RESULTS

### Final weevil population density

Relative to farmer control, ACK reduced final *S. zeamais* population densities on maize seeds at all locations during both seasons (Table 1). During 2010 and 2011 storage period, the treatment reduced weevils by 82 to 85% and 73 to 95%, respectively.

### Seed quality

Relative to farmer-control storage, ACK consistently reduced floating embryo-less seeds with holes by 80 to 97% and 92 to 97% in 2010 and 2011, respectively (Table 2). In contrast, the relative effects of ACK on floating embryo-less seeds without holes was not consistent in 2010 since at Kekana its effect was slightly significant (P ≤ 0.10), but increased and reduced the variable by 34 and 64% at Moletlane and Shatale, respectively. In contrast, during the 2011 season the treatment consistently reduced the variable in all locations by 27 to 80%. Similarly, the treatment consistently reduced sunken embryo-containing seeds with holes by 11 to 70% and 28 to 55% in 2010 and 2011, respectively. In contrast, ACK increased embryo-containing seeds without holes by 101 to 3500% and 945 to 2208% in 2010 and 2011, respectively.

### Seedling vigour

Except for Moletlane in 2010, ACK increased percentage seedling emergence from sunken embryo-containing seeds without holes during the two seasons by 47 to 64% and 27 to 60%, respectively (Table 3). However, ACK had no effect on dry root mass at Moletlane during both years and at Shatale in 2011, but increased the variable in 2010 and 2011 by 29 to 44% and 27%, respectively (Table 4). Also, ACK increased dry shoot mass by 19% but reduced the variable by 33% in 2010 at Kekana and Moletlane, respectively. The treatment had no effect on the variable at Moletlane and Shatale in 2011. Except for Shatale in 2011, ACK increased root/shoot ratios of seedlings at all locations during both seasons.

## DISCUSSION

Use of ACK for seed storage suppressed final *S. zeamais* population densities and improved seed quality and seedling vigour. The mechanism involved in suppression of weevils is intertwined with that involved in improving seedling vigour as viewed under controlled atmospheres

**Table 2.** Relative impact (%) of storing maize seeds in active cattle kraals over farm level storage on mean±SE quality of 500 maize seeds per water-tight container-infested with 20 during two seasons at three locations in South Africa at 120 days after storage.

Treatment	First season, 2010			Second season, 2011		
	Kekana	Moletlane	Shatale	Kekana	Moletlane	Shatale
<b>Floating embryo-less seeds with holes</b>						
Farmer-control	109 ± 2.4	107 ± 2.1	93 ± 4.3	109 ± 2.3	81 ± 1.2	187 ± 4.3
Active-kraal	15 ± 1.2	3 ± 0.2	19 ± 1.3	9 ± 1.8	6 ± 1.3	5 ± 0.9
Impact (%) <sup>y,x</sup>	-86 <sup>*</sup>	-97 <sup>*</sup>	-80 <sup>*</sup>	-92 <sup>*</sup>	-93 <sup>*</sup>	-97 <sup>*</sup>
<b>Floating embryo-less seeds without holes</b>						
Farmer-control	26 ± 3.4	32 ± 4.9	13±2.0	41±3.6	46±2.9	11±0.4
Active-kraal	22 ± 2.0	43±3.2	5±0.8	8±1.2	9±1.8	8±0.7
Impact (%)	-15 <sup>ns</sup>	34 <sup>*</sup>	-62 <sup>*</sup>	-80 <sup>*</sup>	-80 <sup>*</sup>	-27 <sup>*</sup>
<b>Sunken embryo-containing seeds with holes</b>						
Farmer-control	350 ± 10.1	261±11.6	384±20.5	321±11.9	348±19.3	290±6.9
Active-kraal	150 ± 13.8	231±7.9	116±13.4	180±19.8	155±23.8	210±13.5
Impact (%)	-57 <sup>*</sup>	-11 <sup>ns</sup>	-70 <sup>*</sup>	-44 <sup>*</sup>	-55 <sup>*</sup>	-28 <sup>*</sup>
<b>Sunken embryo-containing seeds without holes</b>						
Farmer-control	15±3.1	93±3.9	10±2.2	29±2.7	15±1.9	12±2.7
Active-kraal	313±7.2	222±13.8	360±11.4	303±27.4	345±21.9	277±16.8
Impact (%)	1987 <sup>*</sup>	101 <sup>*</sup>	3500 <sup>*</sup>	945 <sup>*</sup>	2200 <sup>*</sup>	2208 <sup>*</sup>

<sup>y</sup>Impact (%) = [active-kraal/farmer-control] - 1] × 100. <sup>x</sup>Impact values with <sup>ns</sup> and <sup>\*</sup> indicated that treatment means were not significant at P ≤ 0.05 and significant at P ≤ 0.05 according to two-sample Student t-test.

**Table 3.** Impact of storing maize in active cattle kraal over farmer level on mean±SE seedling emergence from 50 sunken embryo-containing maize seeds without holes during two seasons at three locations in South Africa at 120 days after storage.

Treatment	First season, 2010			Second season, 2011		
	Kekana	Moletlane	Shatale	Kekana	Moletlane	Shatale
Farmer-control	34 ± 1.3	43 ± 2.6	28 ± 0.9	39 ± 2.5	37 ± 1.9	30 ± 2.6
Active-kraal	50 ± 3.9	45 ± 1.8	45 ± 2.2	50 ± 3.2	50 ± 4.1	48 ± 1.8
Impact (%) <sup>y,x</sup>	47 <sup>x</sup>	6 <sup>ns</sup>	64 <sup>*</sup>	27 <sup>*</sup>	35 <sup>*</sup>	60 <sup>*</sup>

<sup>y</sup>Impact (%) = [active-kraal/farmer-control] - 1] × 100. <sup>x</sup>Impact values with <sup>ns</sup> and <sup>\*</sup> indicated that treatment means were not significant at P ≤ 0.05 and significant at P ≤ 0.05 according to two-sample Student t-test.

(Hartmann et al., 1988). However, in the cowpea (*Vigna unguiculata*) study, the mechanism through which ACK suppressed cowpea weevils (*Callosobruchus maculatus*) could not be established since seedling vigour was not determined (unpublished data). In both cowpea and maize seeds, anaerobic conditions might have played a role since embryos in seeds have the ability to enter dormancy under anaerobic conditions and successfully emerge from dormancy when anaerobic conditions were ameliorated (Hartmann et al., 1988). Non-significant second and first order interactions suggested that the influence of ACK storage was mainly dependent upon the prevailing conditions within the kraal and at the farm

level. Temperature is known to play a major role in breeding of *S. zeamais*, which requires 15 to 34°C and 40% relative humidity (Danho et al., 2002), while temperatures below 7°C suppress populations of this pest (Nash, 1978).

In ACK, anaerobic conditions are induced in one of two ways: (1) hydrolysis of urine results in the release of ammonia and CO<sub>2</sub> with increased acidic conditions (Bremner and Krogmeier, 1989) and/or (2) acidic and anaerobic conditions favour growth of bacterial populations (Campbell, 1990), which in turn release high concentrations of CO<sub>2</sub>, and therefore, deepening anaerobic conditions. Consequently, in this study we

**Table 4.** Mean±SE dry root mass, dry shoot mass and root/shoot ratio of 30-day-old maize seedlings grown from sunken embryo-containing seeds without holes after 120-day storage at farmer level and under active cattle kraal conditions at three locations in South Africa.

Treatment	First season, 2010			Second season, 2011		
	Kekana	Moletlane	Shatale	Kekana	Moletlane	Shatale
<b>Dry root mass (g)</b>						
Farmer control	2.9 ± 0.48	4.3 ± 0.62	3.8 ± 0.57	3.3 ± 0.78	4.1 ± 0.41	4.1 ± 0.41
Active kraal	4.1 ± 0.95	4.0 ± 0.44	4.9 ± 0.95	4.2 ± 0.41	4.8 ± 0.41	4.4 ± 0.62
Impact (%) <sup>y,x</sup>	44 <sup>*</sup>	7 <sup>ns</sup>	29 <sup>*</sup>	27 <sup>*</sup>	17 <sup>ns</sup>	7 <sup>ns</sup>
<b>Dry shoot mass (g)</b>						
Farmer control	2.1 ± 1.10	3.0 ± 1.34	2.5 ± 0.35	2.7 ± 0.29	2.3 ± 0.11	2.3 ± 0.34
Active kraal	2.5 ± 0.82	2.0 ± 0.36	2.1 ± 0.77	2.3 ± 0.32	2.0 ± 0.09	2.4 ± 0.59
Impact (%)	19 <sup>*</sup>	-33 <sup>*</sup>	-16 <sup>*</sup>	-15 <sup>*</sup>	-13 <sup>ns</sup>	4 <sup>ns</sup>
<b>Root/shoot ratio</b>						
Farmer control	1.38 ± 0.62	1.43 ± 0.38	1.52 ± 0.07	1.22 ± 0.14	1.78 ± 0.28	1.78 ± 0.05
Active kraal	1.64 ± 0.41	2.00 ± 0.11	2.33 ± 0.18	1.83 ± 0.66	2.40 ± 0.27	1.83 ± 0.19
Impact (%)	19 <sup>*</sup>	40 <sup>*</sup>	50 <sup>*</sup>	50 <sup>*</sup>	35 <sup>*</sup>	3 <sup>ns</sup>

<sup>y</sup>Impact (%) = [active-kraal/farmer-control] - 1 × 100. <sup>x</sup>Impact values with <sup>ns</sup> and <sup>\*</sup> indicated that treatment means were not significant at P ≤ 0.05 and significant at P ≤ 0.05 according to two-sample Student t-test.

propose that anaerobic conditions were responsible for the suppression of final population densities of *S. zeamais*, which are invariably aerobic organisms (Campbell, 1990). Commercially, anaerobic conditions are induced in hermetically-sealed containers for seed storage (Hartmann et al., 1988). Similarly, anaerobic conditions are induced under controlled atmosphere (CA) for produce like seeds, vegetables and fruits in order to reduce their metabolic rates, with seeds being stored optimally in CA at 1 to 3% CO<sub>2</sub> and 8% O<sub>2</sub> (Nash, 1978).

The floating test used in this study produced four sets of seeds. Generally, seeds without embryos float, while those with embryos sink to the bottom of the container (Hartmann et al., 1988). Floating embryo-less seeds with holes are those that had the endosperm and embryo completely damaged through infection by *S. zeamais*. In contrast, floating embryo-less seeds without holes were probably harvested with undeveloped embryos, which, due to adversarial storage conditions, especially at the farm level, could not develop further. In contrast, sunken embryo-containing seeds with holes could have been partially damaged, with the weevil activities arrested by unfavourable storage conditions prior to complete damage of the endosperms and their related embryos. Overall, ACK storage improved seed quality and the subsequent seedling vigour, both of which are important in crop propagation (Hartmann et al., 1988; Nzanza et al., 2012).

Generally, the detrimental effect of urea fertilisers on seed germination in soil had been directly linked to ammonia and CO<sub>2</sub>, which are released during hydrolysis

of urea by soil urease (Bremner and Krogmeier, 1989). Apparently, in ACK storage ammonia from urine plays a trivial role since under dormancy seeds are less prone to toxic environments. However, high levels of CO<sub>2</sub> might have played a major role through suppression of respiration, which enhances embryo dormancy.

Seedling vigour in ACK-stored seeds could purely have been due to the preserved growth-promoting substances when seeds entered CO<sub>2</sub>-induced embryo dormancy. However, at the farmer level conditions fluctuated, resulting in embryos using growth-promoting substances. Seeds in the state of deep dormancy are known to result into seedlings with high vigour due to the availability of growth-substances which could have otherwise been used for respiration when the embryo had not entered deep dormancy (Nash, 1978). Increased root/shoot ratios of seedlings from ACK-stored seeds suggested increased root growth at the expense of shoot growth – in support to the above argument. Overall, results suggested that the growth-promoting substances stored in ACK-stored seeds, upon germination, they are channelled towards the proliferation of the radicle and then the root system – resulting in increased vigour of the resultant seedlings. Usually, vigorous seedlings in various crops are associated with higher performance under field conditions (Nzanza et al., 2012).

## CONCLUSION

Use of ACK-storage for maize seeds invariably

suppressed final *S. zeamais* population densities and improved both seed quality and seedling vigour over two seasons. The technology could be useful for storing open-pollinated maize seeds, thereby ensuring the availability of high quality seeds at the farmer level in marginal communities. Incidentally, future studies in ACK-storage are necessary in order to establish: (1) the mechanism involved in suppressing weevil numbers and improving seed quality and seedling vigour, (2) appropriate depth required for optimum storage of seeds, (3) shelf-life of maize seeds stored under this technology, and (4) proper larger storage containers, all of which should be intended to improve food security in marginal communities through seed storage of open-pollinated maize varieties.

## ACKNOWLEDGEMENTS

The authors are grateful to the Land Bank Chair – University of Limpopo, for financial support in the project meant to enhance seed security in marginal communities, the participating farmers for their facilities and the extension officers from the Department of Agriculture, Forestry and Fisheries for technical assistance.

## REFERENCES

- Anon. (2011). Food Security through Seed Security. <http://www.lida.gov.za> (accessed 12 February, 2012).
- Bremner JM, Krogmeier MJ (1989). Evidence that the adverse effect of urea fertiliser on seed germination in soil is due to ammonia formed through hydrolysis of urea by soil urease. *Proc. Natl. Acad. Sci. USA* 86:8185-8188.
- Campbell NA (1990). *Biology*. The Benjamin/Cummings Publishing Company: New York, p. 1163.
- Danho M, Casper C, Haubruge E (2002). The impact of grain quantity on the biology of *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae): Oviposition, distribution of eggs, adult emergence, body weight and sex ratio. *J. Stored Prod. Res.* 38:259-266.
- Gomez KA, Gomez AA (1984). *Statistical Procedure for Agricultural Research*. Wiley: New York, p. 680.
- Hartmann HT, Kester DE, Davies AM Jr., Geneve RL (1988). *Plant Propagation: Principles and Practices*. Prentice Hall: New York, p. 896.
- Hoagland DR, Arnon DI (1950). *The Water-culture Method for Growing Plants Without Soil*. California Agricultural Experimental Station, Circular No. 347, University of California, Berkeley, CA.
- Nash MJ (1978). *Crop Conservation and Storage in Cool Temperature Climates*. Pergamon Press: Oxford, England, p. 393.
- Ngobeni ND (2004). *Effects of Spirostanchys africana on Cowpea Weevil During Seed Storage*. Master Dissertation, University of Limpopo, Sovenga South Africa.
- Ngobeni DN, Mashela PW (2005). Effect of tamboti on weevils on stored cowpea seeds. *Afr. Crop Sci.* 13:381-385.
- Nzanza B, Marais D, Soundy P (2012). Responses of tomato to nursery inoculation with *Trichoderma harzianum* and arbuscular mycorrhizal fungi under field conditions. *Acta Agric. Scand. Sect. B – Soil Plant Sci.* 62:209-215.
- Pierrard G (1986). Control of the cowpea weevil *Callosobruchus maculatus* at the farmer level in Senegal. *Trop. Pest Manag.* 32:197-200.
- Saayman T (1995). Development of practical, low cost methods to control pests and diseases of storage grains. *Plant Prot. News* 42:16-17.
- SAS Institute Inc. (2004). *SAS Online Doc 9.13*, Cary, NC, USA.
- Siwale J, Mbata K, Mrobert J, Lungu D (2009). Comparative resistance of improved maize genotypes and landraces to maize weevil. *Afr. Crop Sci.* 17:1-16.