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Exploiting Biopesticide Potential of Giant African Snail (*Achatina fulica*) Shell against Stored Maize Weevils (*Sitophilus zeamais*) Motschulsky

Adegbola, R. Q[1].,· Jimoh, M. O[1]., Okparavero, N. F[1]., Okunlade, A. F[1]., Akinyera, O. A[2]., Otitodun, G. O[1] ., Haruna P. B[1]., Ishola, T. D[1]., Akande E. J[1]., Odutola B[1]., Sanni L.O[1].

[1]Durable Crops Research Department, Nigerian Stored Products Research Institute, P.M.B 1489, Ilorin, Kwara State, Nigeria
[2]Postharvest Engineering Department, Nigerian Stored Products Research Institute, P.M.B 1489, Ilorin, Kwara State, Nigeria
*Corresponding Author: queensadegbola@gmail.com

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Abstract: *Sitophilus zeamais* is among a wide range of postharvest pest that threatens maize production in Nigeria.Thus, a study was conducted at the entomology laboratory of Nigerian Stored Products Research Institute under ambient condition (30±3°C and 70±6% RH) to assess the biopesticides potential finer powder from *Achatina fulica* shell also known as the giant African snail shell (GASS) against maize storage weevils. The finer powders were applied at dosage rates of 0.25, 0.50, 0.75, and 1.00g/100g of maize grains, and the dosage-mortality effect of GASS powder was investigated at day 7 and 14 respectively while F_1progeny count was done at 47- and 68-days post treatment. The results from the study in table (1) showed a high mean value of (18.67) and (20.00)weevil mortality at dosage level of 1.00g/100g of maize grain within 7- and 14-days post treatment. Mortality of adult insects increased with increase in exposure period and concentration of the GASS finer powders. There was a significant difference in the F_1 progeny of treated maize grain and control (P<0.05). The GASS finer powder at high dosage level (1.00g) inhibited F_1 emergence of the weevil (0.00) in both day 47 and 68 post treatment as shown in table (2). The Control had the highest insect (*Sitophilus zeamais*) infestation, thus leading to high insect damaged kernels (IDK) and the damaged kernel increases with time as shown in table (3). The GASS powder does not have negative effects on the grain germinability as the result shows 100% germinability in maize grain treated with 1.00g of GASS and the control. The result from the study shows that GASS finer powder have considerable bio-insecticides properties and should be incorporated into pest management of stored maize grains since the products are ecological safe.

Keywords: Finer powder, Emergence, Insect damaged kernel, Insecticidal, Mortality, and Progeny.

Introduction

Maize is a versatile crop with environmental adaptation, and it is an important component of agriculture and food systems all over the world (Makate, 2010). Storage of maize is vital in order to sustain constant supply throughout the year for industries and household food supplies as well as to make available seeds for planting (Adetunji, 2007).The Food and Agricultural Organization (FAO,2011) reported that the world wide losses of grains in warehouses are around 10%, but in brazil is about 20% since the storage conditions in the countryside are poor (Gallo *et al.*, 2002). The most common species of insect affecting these maize in warehouses are *Sitopiluszeamais*and this weevil infestation can cause severe damages to stored grains through feeding and also pre-disposes the stored grains to secondary attack by disease-causing pathogens such as mycotoxin-producing fungi; which could lead to increase in respiration and consequent loss in quantity and quality of the grains (Odogola, 1994).*Sitophilus zeamais* infestations cause large losses, and according to Markham *et al*. (1994), *S. zeamays* is becoming more and more of an issue in Africa. Every year, the weevil causes significant losses, and untreated maize has been reported to experience worldwide grain losses of 20 to 90% (Giga *et al.*,1991; Giga & Mazarura,1991). It is necessary to render proper importance to such pest, since cares and expenditure for pest control in field crops would be of no use if the cropped product are being attacked and destroyed when stored.

Currently, fumigation and the use of chemical grain protectants are common methods used to control insect pest infestations in stored grains. However, despite these methods' relative effectiveness, they come with a number of risks, including the emergence of insect resistance to a particular product, pest outbreaks, environmental pollution, pesticide residue, toxicity to plants, people, and non-target organisms, as well as high operational costs (Campos *et al*., 2013; Phillips *et al*., 2000). Despite the success of synthetic pesticides in controlling insect pests, their longevity in the environment, the toxic residues they leave in food and the emergence of insect pest resistance necessitate the search for more low-risk alternatives to the use of chemical pesticides (Ileke& Oni, 2011; Ruiu, 2018).

There are different species of snail meat which are been consumed locally and internationally due to its high protein content and low cholesterol levels. Although various species of snails exists in nature, not all species are good and healthy for human consumption. *Achatinafulica*also known as giant African snail is one of the edible land snails and the demand for its meat has increased in various Nigerians and African restaurants overseas. However, the increase in the consumption of snail's meat has resulted in a high rate of snail shells causing environmental nuisance and pollution if not properly disposed.Finding alternative uses for the giant African snail shell (GASS) is a logical approach to mitigating their negative environmental impacts (Olutoge*et al.*, 2012). Snail shells aresuitable source of chitosan (Abdou *et al.*, 2008) and due to the high content of calcium and magnesium present in the snail shell when synthesized; it may be a grain protectant against major stored grain insect pests. This is because chitosan, calcium and magnesium-rich materials have been shown to control stored product beetle pests (Aremu*et al*., 2023). Therefore, there is an increasing need to convert these wastes (shell) into bio-insecticides products that do not contaminate food when used as grain protectants in small and large-scale storage systems and pose little to no risk to human health and the environment.

This study therefore will be conducted to evaluate the insecticidal potency of giant African snail's shell (GASS) finer powder as grain protectant against stored maize weevil-*Sitophilus zeamais*.

Materials and Methods

Study Site: The experiment was carried out in the entomological laboratory of the Nigerian Stored Products Research Institute (NSPRI), Ilorin, Kwara State.

Sampling of Maize Grains:A stock of untreated commonly grown local white maize (*Zea mays*) grains sourced from identified farmer was used for the study. The maize grain was cleaned by sieving with U.S. Standard #14 sieve (1.4-mm openings) to remove dockage. Damaged kernels were removed and discarded by handpicking. The grain was disinfested by placing in the refrigerator at a temperature of -5°C for seven days in order to eradicate any concealed insect pests. This is due to the fact that all life stages, especially eggs, are extremely sensitive to cold (Koehler, 2003). After seven (7) days, the grains were allowed to equilibrate to ambient temperature and relative humidity until seed moisture content (SMC) reached 13%.

Rearing of Insect Pests:*Sitophilus zeamais* (Coleoptera: Curculionidae) was the species of insect used. Twenty (20) unsexed adult insects were obtained from already existing culture in the insectary and they were introduced into a kilner jar containing 500g of weevil - susceptible maize grains with yeast (500:50, w/w) and covered with muslin cloth. The jar was placed at ambient temperature of 30±3°C and 70±6% relative humidityfor 7 days. Afterwards, the insects were sieved out and only the eggs were left. This procedure allowed obtaining insects with the same age for the study and 5-7 days old insect pests was used.

Purchase and Preparation of Snail Shell:*Achatinafulica* (giant African snail) shell was purchased from an identified snail seller at Ilorin, kwara State. Thereafter, the shell was washed using clean tap running water, dried at room temperature for about ten (10) days and pulverized into fine powder using mechanical grinder. The powder was sieved using a 90µ mesh size to obtain a finer powder and this procedure was adopted as described by Ubong and Godwin (2017).

Toxicity Bioassay

The toxic effect of giant African snail shell (GASS) powder on *Sitophilus zeamais* was accomplished in 150ml of plastic vials containing 100g of maize grains treated with four dosage rate; 0.25g, 0.50g, 0.75g, and 1.00g . Each treatment comprised of three replicates and in each replicate, 100g of maize was treated individually with GASS finer powder making a total of twenty-four (24) replicate. The containers with their contents were gently shaken to ensure thorough coating of the maize grains. Ten (10) freshly emerged (5-7 days old) and unsexed adults *S. zeamais*were introduced into each of the containers and covered with a muslin cloth to allow aeration. Furthermore,synthetic pesticides such as Rambo at 0.17g/100g of maize grain was used as positive control with the addition of ten(10) adult unsexed insect in each of the six (6) replicate plastic vials for day 7 and 14 respectively. Each set up of positive control will have six (6) replicate plastic vialsfor day 7 and 14 respectively.Also, Six(6) replicate plastic vials containing untreated maize grain (with no GASS finer powders but with ten (10) adult insects) was used as control, indicating that, each exposure period will be allocated 3 replicate of untreated maize (control). The plastic vials were kept at room temperature in the laboratory. Mortality count of the weevils was assessed at 7- and 14-days post treatments (here admixing any of the protectants with maize is referred to as "treatment"). Adults were considered dead when probed with sharp objects at the lower abdomen without response (Adedire*et al*., 2011). The percentage insect mortality was calculated using the formular of Niber, (1994):

Where:

$$Percentage(\%) = \frac{Numberofdeadinsect}{Totalnumberofinsect} \text{ x } 100$$

Reduction in progeny production relative to the control was calculated using the formula of Arthur and Throne (2003).

Where:$RPP(\%) = (1 - \left(\frac{NumberofF1treatment}{NumberofF1\,incontrol}\right))x100$

*RPP = Reduction in progeny production

The experimental design for the determination of mortality at 7 and 14 days, 47- and 68-days progeny production was a Randomized Complete Block Design (RCBD), with sub-sampling (3 jars associated with each replication comprised subsamples).

The maize seeds were reweighed; the percentage weight loss was determined and recorded using the formula described by Odeyemi and Daramola (2000).

Where:

$$Percentage(\%)Weightloss = \frac{Initialweight - finalweight}{Initialweight} \text{ x } 100$$

Germination Test:

Grain germination test was determined days after progeny count. After the F1 progeny count, 10 grains were randomly picked from each sub-sample per treatment, and the grains wereplaced on moistened cotton wool in 9 cm diameter disposable petri-dish which was humidified once in every 2 days. Germination count was taken on the 12th day (Rao *et al*., 2006).

Percentage germination was calculated using the formula described by Adedire*et al* (2011).

Where: $Percentage(\%)Germination = \frac{Numberofgerminatedseeds}{Totalnumberofseedsplanted} \text{ x } 100$

Data Analysis

All the tests were performed with three independent replicates, and the data was expressed as mean values with standard error of mean and percentage. A repeated measure ANOVA at 95% confidence interval was conducted to examine the effect of the treatments and time on different parameters using IBM SPSS statistical package (SAS Institute, 2013).

Results and Discussion

Insect Mortality

The result of the experiment in table (1) and figure (1) below, shows that there was a significant difference in the main effect of treatments and time on the number of insect mortality ($p \geq 0.05$) at day 7, when compared with the treated and untreated maize grain.However, at day 14, there was no significant difference ($p<0.05$) between the high dosage level (1.00g) of GASS and Rambo™. These indicate that GASS is as effective as Rambo™ a commonly used synthetic pesticide. Furthermore, the maize grain treated with 0.75g and 1.00g of GASS powder generated high mortality rate with a mean value of (18.67) at day 7 and (19.33) and (20.00) at day 14 respectively; when compared to Rambo, a synthetic pesticide (positive control). It was observed that the higher the dosage level, the higher the death rate and the deathrate also increases with time.

Table 1: Mean effect of treatmenton insects' mortality

Treatment	Mean±S.E	
	7 days	14 days
Shell 0.25	17.33±0.577^{a}	17.67±0.882^{a}
Shell 0.50	18.00±0.882^{a}	18.00±0.577^{ab}
Shell 0.75	18.67±0.333^{a}	19.33±0.333^{ab}
Shell 1.00	18.67±0.333^{a}	20.00±0.000^{b}
Rambo™	17.00±1.155^{a}	19.67±0.333^{b}
Control	0.25±0.333^{b}	1.67±0.333^{c}

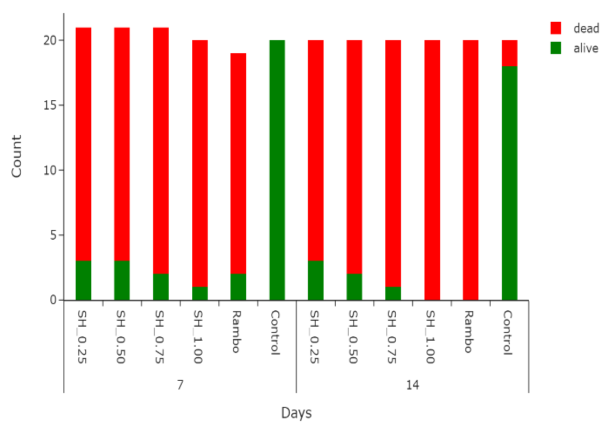


Fig 1: A bar chart showing the mean effect of treatment on insect'smortality

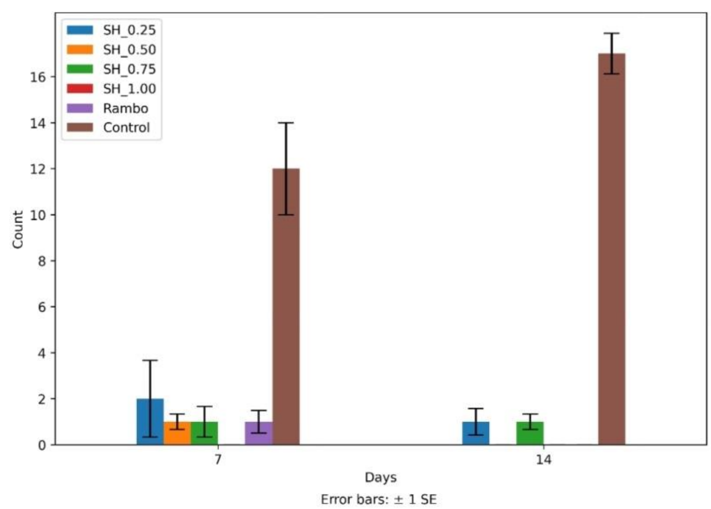
F_1Emergence Count

After seven (7) days addition of the insects to the treated and untreated maize, contents (maize) from the 3 plastic vials earlier assessed for mortality was sieved to remove all insects (dead and alive), and the plastic vials were closed with muslin cloth and left undisturbed in the laboratory for an additional 40 days (47 days post treatment) before F1 progeny in each vials was determined. The same procedure was assigned to the remaining 3 vials assessed for mortality at 14 days post treatment and left undisturbed in the laboratory for an additional 54 days (68 days post-treatment) before the number of F_1 emergence in each of the vials was determined (Nwaubani, 2006).

From the results shown in table (2) and graph (1) below, it was observed that there was a significant difference in F1 progeny count between the treated and untreated maize grain ($P \geq 0.05$). The untreated maize grain (control) had the highest count of live insect with a mean value of (12.00) at day 47 post treatment and the progeny count increases with passage of time showing a mean value of (16.67) at day 68 post treatment respectively. Additionally, maize grain treatedwith low dosage level of GASS powder (0.25g) also show live insectwith a mean value of (1.67) at day 47 and (1.00) at day 68. Due to the effectiveness of the GASS powder, the emergence count of the weevils was significantly reducedat high dosage level of1.00g with a mean value of (0.00) in both 47- and 68-days post treatment.Furthermore, the F1 progeny decreases with time in the treated maize grain but increases with time in the untreated maize grain (control). Hence, there was no significant difference ($p<0.05$)in the level of progeny count in day 47 and 68 respectively.

Table 2: Mean effect of treatment count on F_1 emergence with time

Treatment	Mean±S.E	
	7 days	14 days
Shell 0.25	1.67±0.577^{a}	1.00±0.882^{a}
Shell 0.50	0.33±0.882^{a}	0.00±0.577^{a}
Shell 0.75	0.67±0.333^{a}	0.33±0.333^{a}
Shell 1.00	0.03±0.333^{a}	0.00±0.000^{a}
Rambo™	0.50±1.155^{a}	0.00±0.333^{a}
Control	12.00±0.333^{b}	16.67±0.333^{b}



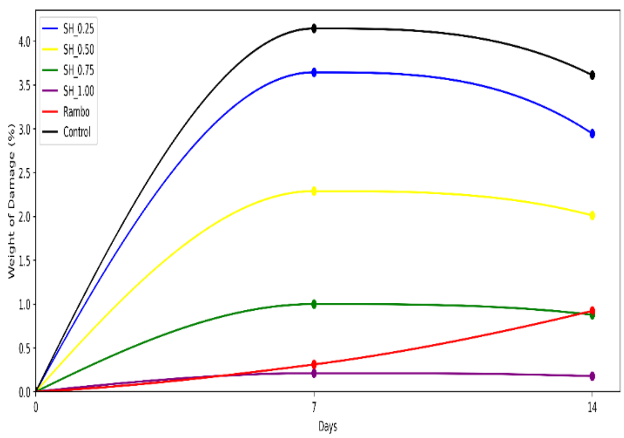
Graph 1: Showing the mean effect of GASS powder treatment on F_1emergence count

Insect Damaged Kernel (IDK)

From the result shown in table (3) and graph (2) below, there was a significant difference in the main effect of treatments in the occurrence of insect damaged kernel on the treated maize grains ($p>0.05$). Maize grain treated with 1.00g had the least insect kernel damaged with a mean value of (1.17) and (0.33) in day 7 and day 14 respectively. Due to the effectof insect kernel damaged on the grain, the weight loss on the grain reduces with time. However, the control maize grain with no treatment, had the highest kernel damage (4.56)in day 7 and (5.89) in day 14 respectively; followed by maize grain treated with 0.25g of GASS powderwith a mean value of (3.56)kernel damage in day 7 and (3.11) in day 14. The results ascertained that the GASS powder was effective since no significant difference was observed in the kernel damage on the high dosage level when compared to Rambo^{TM}, a synthetic pesticide. Furthermore, the weight loss on the maize grain reduces as the dosage level of treatment on the maize grain increases. However, when compared with time, the kernel damage increases with the passage of time.

Table 3: Mean effect of treatment on quantity of insect damaged kernel

Treatment	Mean±S.E	
	7 days	14 days
Shell 0.25	3.56±0.444^{a}	3.11±0.484^{a}
Shell 0.50	1.87±0.556^{ab}	1.22±0.909^{ab}
Shell 0.75	1.56±0.484^{ab}	1.42±0.401^{b}
Shell 1.00	1.17±0.167^{b}	0.33±0.333^{b}
Rambo^{TM}	1.44±0.111^{b}	1.78±0.778^{b}
Control	4.56±0.988^{a}	5.89±1.829^{a}



Graph 2: Showing the mean effect of treatment on the weight loss of insect damaged kernel

Germinability

The result in table (4) and figure (2) below shows that there was no significant difference ($p<0.05$) in the germinability status of the maize grain treated with GASS powder especially at dosage level of 1.00g (100%) when compared with the untreated maize grain (control) and Rambo^{TM} (positive control).

Table 4: Percentage effect of treatments on germinability of maize grains

Treatment	Percentage (%) ± S.E
shell_0.25	98.33±1.667^{a}
shell_0.50	98.33±1.667^{a}
shell_0.75	98.33±1.667^{a}
shell_1.00	100.00±0.000^{a}
Rambo^{TM}	95.00±2.887^{a}
control	100.00±0.000^{a}

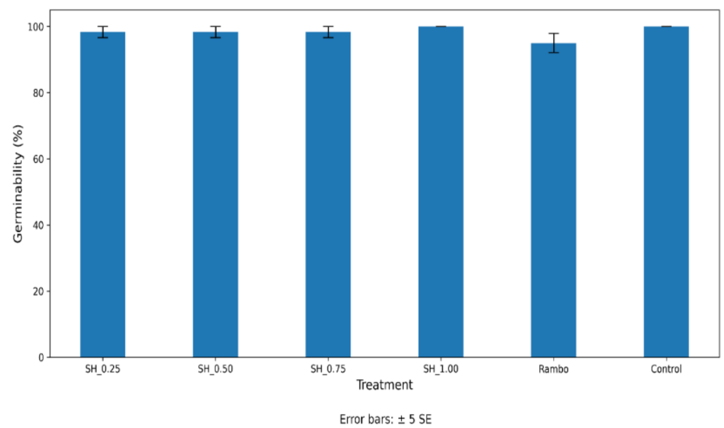


Fig 2: A bar chart showing the percentage of maize grain germinability

Achatinafulica shell which was used in this study as a grain protectant against maize weevils has an elongated hollow cone, which is spirally coiled round a central axis called columella (Yoloye, 1994). The thickness and degree of mineralization of thesnail shell increases with the age of the snail as well as age of the shell whorls (Barker, 2001). The shell has a brownish yellow background with fairly uniformly arranged bands and zigzag lines or spot that are dark brown or reddish brown in colour. The apex of the shell is slightly flattened, bulbous and pale or pinkish in colour. These differentiate them from other varieties of snail's shell and they are widely distributed (Akinnusi, 2002).

Akinnusi, (2002) reported that snail shells have been used extensively in traditional medicine to treat measles, cough and gonorrhea.Snail shell is a suitable source of chitosan (Abdou*et al*., 2008) and due to the high content of calcium and magnesium present in the shell, it is used in wound healing process; a prerequisite for blood clotting process. Houndonougbo*et al*., (2012) opined that in animal husbandry, the shell is used in feed formulations for poultry and other livestock. Amubode and Fafunwa (2014) likewise reported on the uses of snail shells for aesthetic purposes in cars, homes and offices. Furthermore, researchers in Nigeria have listed 15 health conditions that are believed to be curable with the meat, fluid and shell of giant African snails (Osemeobo, 1992). In fact, shell by-products have been used as a partial substitute for cement, sand or gravel for mortars and concrete (Othman, *et al*., 2013; Muthusamy *et al.*, 2012; Olivia *et al*., 2015; Nguyen *et al*., 2016; Yang *et al*., 2005; Cuadrado*et al*., 2015 and Woon*et al.*, 2015). Although, there are very few information on giant African snail shell finer powder, being used as grain protectant against stored product insect pests, but there are lot of information on chitin, chitosan synthesized from sea food such as crab shell and was reported effective against stored product insect pest of maize and cowpea (Otitodun*et al.*, 2015; Sahab, *et al.*, 2015). Houndonougbo, (2012) reported that *Achatinafulica*shell is effective as grain protectant against insect pest of maize because of the presence of chitin and chitosan in the shell when synthesized and its high calcium carbonate content. Laboratory experiment conducted at the food Technology Research and Training center (FTRTC) at Tambon (Indonesia) have shown that calcium carbonate powder achieved effective control of *Triboliumcastaneum* (herbst) in stored paddy for a period of nine month (Gahukar, 1994). Chitosan synthesized from snail shell has unique biological activities such as biocompatibility (Hsu *et al.,* 2011; Mi *et al*., 2002), biodegradable (Kim *et al.,* 2011), nontoxicity (Shi *et al.,* 2006), antimicrobial activity (Li *et al.*, 2008; Rabea *et al.,* 2009), antitumor activity (Toshkova*et al.*, 2010) and immune- enhancing effect (Li *et al.,* 2013c; Zaharoff*et al.,* 2007). These properties make chitosan a promising entrant for medicine (Tan *et al.*, 2013), food (Dutta *et al.*,2009; Qiu*et al.*, 2014) cosmetics (Ray, 2011), water treatment (Bhatnagar and Sillanpaa 2009) and biomedical engineering industries (Silva *et al.*, 2012; upadhyaya*et al.*, 2013) as well as for many agricultural uses (Cota-Arriola*et al* 2013; El-Hadrami*et al.*, 2010). Chitosan proved antimicrobial activities against fungi, bacteria and viruses and act as an elicitor of plant defense mechanism; prevent the spread of pathogens (Li and Zhu2013; Mansilla*et al*.,2013) and enhance plant innate immunity defenses (Amborade*et al.,* 2008; Fondevilla and Rubiales 2012). All these properties show that giant African snail shell can be a promising entrant for the control of insect pest of stored produce.

Conclusion and Recommendation

Agricultural produce has met with reduced prices, because of insect infestation. Presently, fumigation and the application of synthetic chemical grain protectants are measures commonly used to control insect pest infestation in stored grains. However, the maize grain needs to be stored using effective and safe postharvest management measures to prevent physical insect damage. Therefore, there is an increasing need for furthers research to be done on thechitin and chitosan synthesized from *Achatinafulica* shell since its finer powder has proved to be relatively effective against maize weevils. The shells are locally available, relatively safe and edible product that will poses little or no hazard to human health and the environment and will not contaminate food product in acting as grain protectant in small- and large-scale storage systems.

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Ruiu Luca (2018):Dipartimento di Agraria, University of Sassari, 07100 Sassari, Italy; lucaruiu@uniss.it; Tel.: +39-079-229-326 2 BioecopestSrl, Technology Park of Sardinia, 07041 Alghero (SS), Italy

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