Available online at http://www.ifg-dg.org

Int. J. Biol. Chem. Sci. 10(1): 242-254, February 2016

ISSN 1997-342X (Online), ISSN 1991-8631 (Print)

International Journal of Biological and Chemical Sciences

Original Paper http://ajol.info/index.php/ijbcs http://indexmedicus.afro.who.int

Production of freshwater benthic macroinvertebrates from pig dung: fertilization effect and optimal dose research

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ACKNOWLEDGEMENTS

The authors are grateful to the Scientific Council of Abomey-Calavi University in Benin for funding the present research in the framework of OPASISI (Agricultural Production Optimization Without Inputs Integrated System) project.

ABSTRACT

Different pig dung doses were used to fertilize medium production of freshwater benthic macroinvertebrates in order to test the fertilization effect and determine the optimal dose. In fact, the experiment was carried out in triplicate, for 42 days in plastic buckets. These buckets were grouped in seven treatments (T_1, T_2, T_3, T_4, T_5, T_6, and T_7) which were fertilized (respective doses of 5%, 10%, 15%, 25%, 50%, 75% and 100% with pig dung in relation to the total substrate volume) and a control (T_0) which was not fertilized (0% of pig dung). Each bucket was seeded in benthic macroinvertebrates with an initial density of 9 individual/dm^3 (D_0). The results revealed that the utilization of pig dung improved the water chemical properties in the production medium as well as the macroinvertebrates density ($p < 0.05$). The optimal production of the latter was obtained with the dose of pig dung applied to treatment T5 (50%), that is 150 g of dry dejections per dm^3 of substrate (150 g/dm^3) with a total average density of 742 ± 569 individual/dm^3. The treatment T_5 (50%) constitutes then the dry pig dung optimal dose to be recommended for benthic macroinvertebrates production.

Keywords: Fertilization, macroinvertebrates, optimal dose, pig dung, production**.**

INTRODUCTION

Benthic macroinvertebrates are part of aquatic organisms using as live foods to other living beings found in the aquatic ecosystems; there were very important for the nutrition of many fish species (Wais et al., 1999; Evangelista et al., 2005). The insects and annelids played a decisive role in the food chain of aquatic ecosystems as they were most consumed by fish. In fact, the Chironomidae

DOI : http://dx.doi.org/10.4314/ijbcs.v10i1.19

(Diptera) represented the benthic aquatic insects most abundant in fresh water ecosystems (Wetzel, 2001) whereas the Tubificidae (oligochets) constituted an excellent live food for the fish rearing (Evangelista et al., 2005). These benthic macroinvertebrates were excellent source of proteins of lipids, vitamins and mineral salts (Thipkonglars et al., 2010; Aslianti et al., 2011). They have a big breeding capacity through their high fecundity, a generation time relatively short and expand over a wide range of organic substrate. They were poluorésistants as they tolerated unfavorable variations of most environmental factors. Their development can be influenced by numerous environmental factors such as temperature and photoperiod, the availability and quality of food (Vos et al., 2000).

The macroinvertebrates were also used as live food for carnivorous fish fry in pisciculture because their larvae constituted basic foods in the ration of almost all those young fish (Das et al., 2012). Thus, the rearing of *Clarias fuscus* and *Clarias macrocephalus* fry with benthic macroinvertebrates has given good growth performance (Evangelista et al., 2005). This importance of the live prey in the fish rearing has increased the interest of their production in the laboratory (closed system/controlled) in the most of the cases. However, production in laboratory requires high financial resources. Furthermore these studies, conducted in the laboratory, were often monospecific, constraining and required specialists (Tavares et al., 2009).

These works of macroinvertebrates production were often made in fertilized medium with organic matter. Among the organic fertilizers, there were plant wastes such as: rice and wheat bran, soya flour, mustard cake, coconut fibers and lettuce and animal dejections such as poultry droppings, cow dung, sheep and horse dejections (Hossain et al., 2011) and finally, pig dung which were rarely used. In fact, the organic fertilizers (animal manures) favored a good growth of macroinvertebrates population. However, their excessive utilization, without control, could provoke the fall of organisms density; hence the use of an optimal dose (Adedeji et al., 2011). It is therefore necessary to use the optimal quantity of these different dejections per unit of volume so as to permit a good macroinvertebrates production.

In Benin, to our knowledge, no research has been made on the benthic macroinvertebrates production with organic fertilizers and precisely with pig dung. That is what justifies the present study which aimed at testing the effect of fertilization from pig dung on the freshwater benthic macroinvertebrates plurispecific production and at determining these dejections optimal dose.

MATERIALS AND METHODS

Experimental plan

The experimental plan was constituted of 24 plastic buckets, 80 liters of capacity, disposed at free air at the research station on pisciculture diversification in Research Laboratory on Wetlands (LRZH) at the University of Abomey-Calavi (UAC). They were grouped into 7 treatments (T_1, T_2, T_3, T_4, T_5, T_6 and T_7) and a control (T_0) in 3 replicate per treatment; the control was also replicated thrice. Before the experience starts, the buckets were cleaned with bleach water and rinsed abundantly and dried for 24 hours. The next day, each bucket received 10 dm^3 of substrate over which were respectively poured 16 liters of drilling water and 4 liters of pond water green enough (Madlen, 2005) filtered with a 200 µm silk. The substrate was constituted of dry pig dung mixture and sand, previously sieved with a 1 mm metallic sieve, at different proportions. The dejections used come from pig fed with Azolla, rice bran and palm oil. They contained 47.05% of dry matters, 68.91% of organic matters, 31.09% of mineral matters; 1.43% of total nitrogen, 1.60% of total phosphore and 0.77% of total potassium. Then the treatments T_1, T_2, T_3, T_4, T_5, T_6 and T_7 received respectively a supply of 5%, 10%, 15%, 25%, 50%, 75% and 100% of pig dung in relation to the substrate total volume; the control was not fertilized (0% of dejections). Which corresponds to respective doses of 15 g/dm^3; 30 g/dm^3; 45 g/dm^3; 75 g/dm^3; 150 g/dm^3; 225 g/dm^3 and 300 g/dm^3 of dry pig dung per substrate. All the buckets were covered with a mosquito net.

Three (03) days after the fertilization, the buckets were seeded in fresh water benthic macroinvertebrates harvested in the pond of research on pisciculture diversification station of LRZH (UAC) with a 500 µm net. Each bucket was seeded in macroinvertebrates with 1 dm^3 of that mud and then an under-sampled of 0.1 dm^3 was formaldehyde (10%) for the counting and the identification of different macroinvertebrates groups observed with a binocular loupe. Then, at a 7 ind/dm^3 seeding rate of Chironomidae and 2 ind/dm^3 of mollusk in each bucket that is a total of 9 ind/dm^3 of macroinvertebrates per bucket.

Physico-chemical parameters

During the experiment, the culture medium physico-chemical parameters (pH, conductivity, temperature and dissolved oxygen) of water were measured *in situ,* once a week. The pH, conductivity and the temperature were measured with a multi-parameter conductimeter (HI 9143 Microprocessor Auto Cal Dissolved Oxygen Meter). Diverse water chemical analyses of each production medium were then performed with 500 ml of water taken in the plastic bottles (0.5 l of capacity). Then, the ammonium, the nitrates, the nitrites and the phosphates were respectively dosed by Nessler-380 methods, of reduction with Cadmium-335, of Diazotation-371 and of Phosver3-490 with HACH spectrophotometer.

Macroinvertebrates production follow-up

The macroinvertebrates were sampled every seven (07) days after the seeding and this for 42 days (D_{42}). In each bucket, 0.1 dm^3 of substratum taken and sieved with a 500 µm sieve; they were fixed with formaldehyde at 10%. The present individuals were isolated, identified and counted with a binocular loupe in order to know the densities of diverse groups. They were afterwards conserved in alcohol (ethanol) at 70%.

Statistical analyses

The statistical analysis of obtained results was performed with the statistics software SAS version 9.2 by analysis of variance method with one classification criteria (ANOVA 1) (Sherrer, 1984; Dagnelie, 1984). The LSD (Least Significant Difference) of Fisher (Saville, 1990) was used to compare the different means. A p value < 0.05 was considered statistically significant.

RESULTS

Physico-chemical parameters variation

The physico-chemical parameters mean values in the different treatments culture medium are summarized in Table 1. According this table, the mean temperature of water in buckets was around 30.17 ± 0.39 °C. The pH mean values in fertilized medium were around 7.43 ± 0.22 °C and slightly fluctuated whereas they were weak in the control (6.38 ± 0.12 °C). The dissolved oxygen mean values high in the control buckets (5.38 ± 0.43 mg/l) was around 4.33 ± 0.32 mg/l in treatment medium T_1 and T_5 and were low in those of treatments T_6 and T_7. The conductivity and concentration means of NH_4^+, NO_2^-, NO_3^- and PO_4^{3-} increased regularly with the different doses of pig dung. They were higher in fertilized buckets with 100% of dejections (T_7) and lower in control treatments.

The variance analysis with only one criteria (ANOVA 1) applied to the different parameters values (Table 1) revealed significant differences of the conductivity and the ammonium, nitrites, nitrates and phosphates rates between the fertilized medium and the different dejection doses ($p < 0.05$).

Macroinvertebrates

The benthic macroinvertebrates groups identified in this study were mollusk (12%) and the Chirominidae (88%) which were more abundant. The total mean densities of macroinvertebrates were more important in fertilized medium in relation to the control. But they experienced an increase with the pig dejection doses in all the culture medium, during the experiment. In fact, the higher densities were obtained in the buckets of treatments T_5, T_6 and T_7; whereas the lower ones were observed in treatment medium T_2, T_3 and T_4; those densities were very low in treatment T_1 buckets (Figure 1). The most important macroinvertebrates density was observed in the treatment T_7 (852 ± 846 ind/dm^3) which was followed by treatment T_6 (753 ± 637 ind/dm^3) and then finally by T_5 (742 ± 659 ind/dm^3). The variance analysis with only one criteria (ANOVA 1) revealed that there was no significant difference between the macroinvertebrates average densities in treatment T_5 and T_6. Likewise, there was no significant difference in treatment T_1 and the control ($p > 0.05$).

According to Figure 2, the macroinvertebrate mean densities in non-fertilized medium (control) and those of treatment T_1 (5% of dejections) reached their peak at 7th production day (D_7); whereas those of treatment T_2 (10% of dejections) reached their peak at 14th production day (D_{14}). The other treatments (T_3, T_4, T_5, T_6 and T_7) reached their peak on 21st production day (D_{21}). These maximal densities increased with the different dejection doses. The most important maximal density was obtained with treatment T_7 (2097 ind/dm^3) followed by the one in treatment T_6 (1717 ind/dm^3). Let's point out that on 21st production day (D_{21}), the treatment medium T_5 (50% of dejections) had a mean density (1483 ind/dm^3) which was slightly inferior to the one of 28th production day (1353 ind/dm^3). That density was then more or less constant between D_{21} and D_{28}, contrarily to the fertilized medium with the other doses where the density falls just after their peak.

The highest mollusk mean densities were obtained in treatments T_5 and T_6 buckets; whereas they were low in treatments T_2, T_3 and T_4 medium and very low in treatments T_1 and T_7 medium (Figure 3). Then the mollusk mean density was more important with treatments T_5 (82 ind/dm^3) followed by the one of treatment T_6 (77 ind/dm^3). The variance analysis with one classification method (ANOVA 1) revealed that there were no significant differences between the mollusk mean densities in treatments T_5 and T_6. Likewise, there were no significant differences between treatments T_1 and T_7 and the control ($p > 0.05$).

Chironomidae mean densities in fertilized medium with different pig dejections doses know an increase with the doses, in all the culture medium during the experimentation. In fact, the highest densities were obtained in treatment T_5, T_6 and T_7 buckets; whereas they were low in treatments T_2, T_3 and T_4 medium and then very low in treatments T_1 (Figure 4). Therefore, the Chironomidae mean density the most important was observed with the treatment T_7 (829 ± 840 ind/dm^3) followed by the one of treatment T_6 (679 ±588 ind/dm^3) and at last the one of treatment T_5 (660 ± 509 ind/dm^3). The variance analysis with one criteria (ANOVA 1) revealed that there was no significant differences between the macroinvertebrates mean densities in treatments T_5 and T_6. Likewise, there were no significant differences between treatment T_1 and the control one ($p > 0.05$).

The Chironomidae mean densities reached their peak the 21st production day (D_{21}) in treatments T_3, T_4, T_5, T_6 and T_7 with the predominance of treatment T_7 density (2097 ind/dm^3) followed by the one of treatment T_6 (1567 ind/dm^3). But the densities of these Chironomidae in treatment T_5 medium (fertilized with the dose 50%) slightly diminished at D_{21} (1320 ind/dm^3) at D_{28} (1223 ind/dm^3). That macroinvertebrates group is then more or less constant between D_{21} and D_{28} to that dose. On the other hand, these organism densities have fallen with the other treatments during the same period (Figure 5).

Table 1: Different treatments physico-chemical characteristics.

	T_0	T_1	T_2	T_3	T_4	T_5	T_6	T_7
Temperature (°C)	30,97 ± 1,96[a]	30,52 ± 2,22 [b]	30,24 ± 2,33 [c]	29,95 ± 2,32 [d]	29,86 ± 2,41 [d]	29,88 ± 2,56 [d]	30,02 ± 2,56 [d]	29,96 ± 2,56 [d]
pH	6,38 ± 0,12 [a]	7,15 ± 0,16 [b]	7,5 ± 0,10 [c]	7,70 ± 0,18 [e]	7,66 ± 0,27 [e]	7,50 ± 0,27 [c]	7,37 ± 0,31 [d]	7,17 ± 0,24 [b]
dissolved oxygen (mg/l)	5,38 ± 0,43 [a]	4,71 ± 0,26 [b]	4,47 ± 0,32 [c]	4,46 ± 0,23 [c]	4,12 ± 0,59 [d]	3,91 ± 0,57 [e]	3,16 ± 0,21 [f]	2,52 ± 0,22 [g]
conductivity (µS/cm)	313,31 ± 23,51[a]	546,76 ± 48,92[b]	797,76 ± 77,06 [c]	868,33 ± 68,55 [d]	1192,86 ± 107,74 [e]	1824,69 ± 110,07 [f]	1926,36 ± 53,22 [g]	2042,48 ± 37,94 [h]
NH_4^+ (mg/l)	2,512 ± 0,013 [a]	2,958 ± 0,005 [b]	4,246 ± 0,02 [c]	4,912 ± 0,007 [d]	5,731 ± ,0005 [e]	13,775 ± 0,006 [f]	21,020 ± 0,027 [g]	35,435 ± 0,014 [h]
$N0_2^-$ (mg/l)	0,021 ± 0,0002[a]	0,028 ± 0,0002[b]	0,041 ± 0,0007 [c]	0,054 ± 0,0006 [d]	0,066 ± 0,00003 [e]	0,076 ± 0,0006 [f]	0,123 ± 0,001 [g]	0,134 ± 0,001 [h]
$N0_3^-$ (mg/l)	4,683 ± 0,006 [a]	4,838 ± 0,142 [b]	7,535 ± 0,009 [c]	9,852 ± 0,008 [d]	12,163 ± 0,009 [e]	17,522 ± 0,01 [f]	36,300 ± 0,0136 [g]	40,054 ± 0,065 [h]
$P0_4^{3-}$ (mg/l)	0,582 ± 0,007 [a]	0,914 ± 0,009 [b]	1,81 ± 0,008 [c]	3,082 ± 0,01 [d]	4,634 ± 0,01 [e]	7,714 ± 0,009 [f]	10,17 ± 0,019 [g]	21,875 ± 0,036 [h]

The values affected with the same letter in exponent on the same line were not significant different ($p > 0.05$).

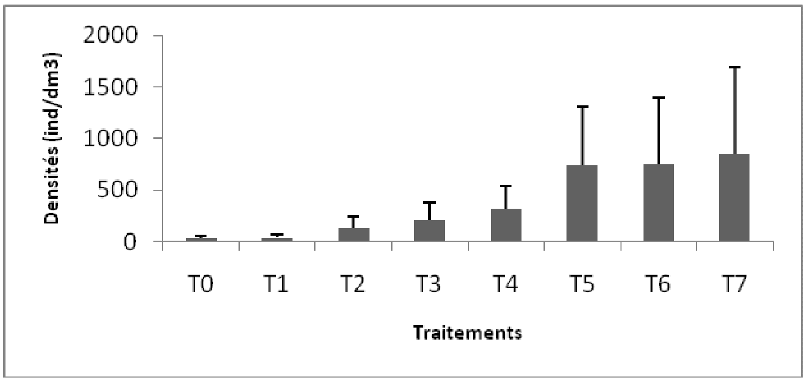

Figure 1: Benthic macroinvertebrates total densities by treatments.

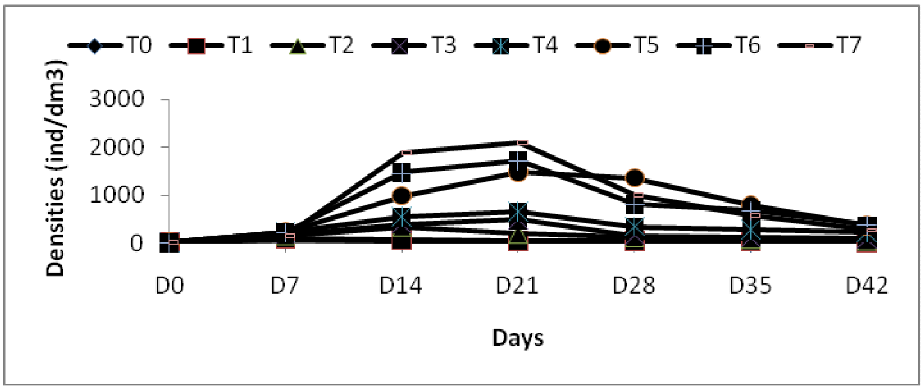

Figure 2: Evolution of the benthic macroinvertebrates total densities in different treatments in function of time.

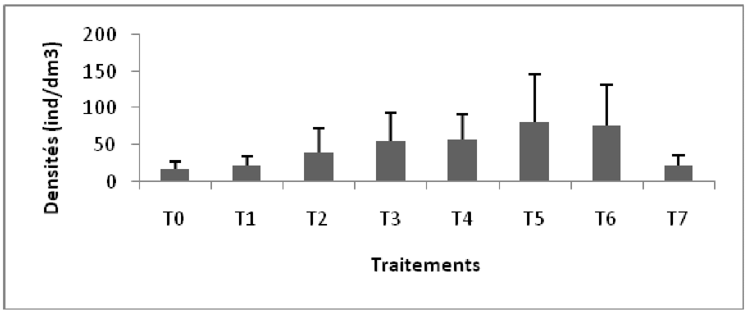

Figure 3: Mollusk total densities by treatments.

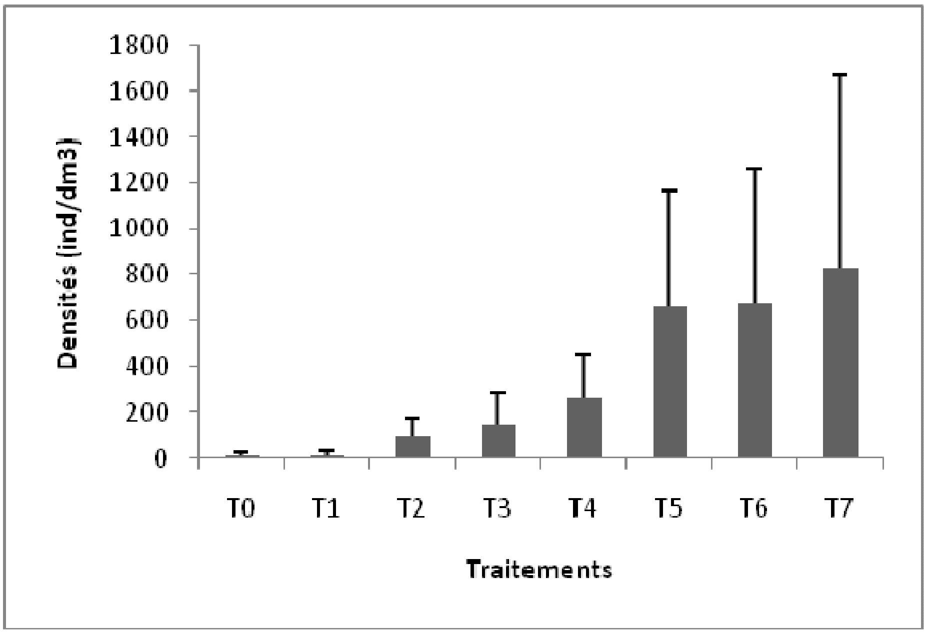

Figure 4: Chironomidae total densities in different treatments in function of time.

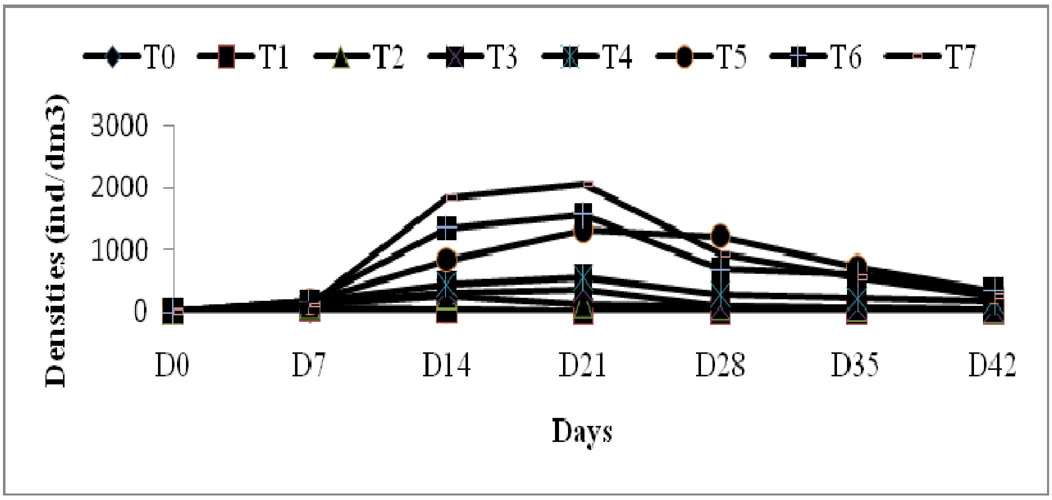

Figure 5: Evolution of the Chironomidae total densities in the different treatments in function of time.

DISCUSSION

Physico-chemical parameters

Pig dung offer satisfactory conditions for the fresh water benthic macroinvertebrates plurispecific breeding. They allowed a supply in nutrients in the breeding medium; thus the concentrations in ammonium ions (NH_4^+), phosphate ions (PO_4^{3-}), nitrate ions (NO_3^-), nitrites ions (NO_2^-) were higher in fertilized buckets and were significantly different from the control buckets (non-fertilized). These results were comparable to those obtained by Dhawan and Kaur (2002), Parvez et al. (2006), Tavares et al. (2009) and Akodogbo et al. (2014a) which showed that animal dejections utilization as fertilizers improved the water physico-chemical properties. That enrichment of fertilized medium in nutritious salts was due to the liberation of minerals by the organic matter (pig dung) following their mineralization (Bokossa et al., 2014). Pig dung provided the necessary nutrients to an important primary production (Akodogbo et al., 2014a).

But the water physico-chemical quality changes according to the quantity of fertilizers introduced in the medium (Pratap et al., 2005). Then the conductivity and the dissolved salts rates of water in the different culture medium increased with different pig dejections doses used. These results were comparable to the ones obtained by Akodogbo et al. (2014b) in fertilizing the culture medium with pig dung and then by Adedeji et al. (2011) who used chicken droppings.

Globally speaking, physico-chemical parameters of macroinvertebrates production medium allowed the Chironomidae development as these organisms have a tolerance raise in environment unfavorable conditions. Mean temperature (30.17 ± 0.39 °C) was conformed to the one of good production (27.9 to 30.2 °C) of Chironomidae larvae (Sulistiyarto et al. 2014); for temperature is one of the major factors that control the growth rate and aquatic insects development (De Haas et al., 2006; Ozkan and Elipek, 2007). The dissolved oxygen rates in different treatments were in norms for the Chironomidae development, for the latter can live in medium where the oxygen rate is inferior to 2 mg/l (Sulistiyarto et al., 2014). The water pH values in fertilized medium (7.43 ± 0.22) were up to those obtained by Kumar and Ramesh (2012) during the Chironomidae rearing with organic materials (6.8 to 7.5).

Benthic macroinvertebrates production

Benthic macroinvertebrates production depends not only on the quality of water (Ozkan and Elipek, 2007; Wulandari et al., 2005) but also on the quantity of available food (Olivera et al., 2003; De Haas et al., 2006; Solomon et al., 2008). In fact, the one obtained in fertilized bucket with pig dung was clearly better than the controls one. This is easily explained by the fact that the utilization of organic fertilizers has a positive impact on the abundance of living organisms (Kang'ombe et al., 2006).

Macroinvertebrates high densities in treatments medium T_5, T_6 and T_7 were explained by the high doses of pig dung in those medium contrarily to the other treatments which received low doses of that fertilizer. The Chironomidae abundance in these macroinvertebrates culture is linked to the substratum nature; for the organic matter in decomposition (detritus) was the main source of food of these organisms (Callisto et al., 2007; Sanseverino and Nessimian, 2008). In the other hand, the mollusk generally feed themselves with microphytes (Tachet et al., 2009). The Chironomidae density was more important in treatment T_7 medium for they received more food than the others. This confirms the works of Sulistiyarto et al. (2014) which showed that the best production results of Chironomidae larvae were provided by the substratum that had the highest ability to alimentary trapping. The fertilization has then a positive impact on the macroinvertebrates production, for the production medium having received low doses gave low productions. This confirms the works of Kumar and Ramesh (2012) which showed that Chironomidae production increases in crescent way with the rise of cow dung doses (0.05; 0.10 and 0.15 g/l). But the very weak doses of that fertilizer don't allow these organisms development; likewise, the very high ones have a bad consequence for the optimal production.

Thus, the threshold pig dung dose for the fresh water benthic macroinvertebrates production was 10% (T_2) in relation to the substratum volume; for it's from this dose that the fertilization effect was significantly remarkable. In fertilized medium with dejection doses 75% and 100% (respectively treatments T_6 and T_7), the macroinvertebrates maximal density fall on 21st day has shown that the environmental conditions were difficult from that day. This was explained by the high rates of mineral substances in the culture medium. These pig dung doses don't allow then an optimal production of benthic macroinvertebrates. Yet, with the dose 50% (T_5), the macroinvertebrates density was slightly constant between the 21st and 28th production day (D_{21} to D_{27}). The environmental conditions of fertilized medium with that dose were then favorable to these organisms good development. Likewise, the mollusk total mean density was more important with that dose. The latter permitted then to maintain the densities of the different macroinvertebrates groups in the production medium.

In the light of the foregoing, the optimal dry pig dung dose for the plurispecific and optimal fresh water benthic macroinvertebrate production was 50% of dejection in relation to the substratum volume (T_2), that is 150 g of dejection per dm^3 of substratum (150 g/dm^3). It allowed to produce different macroinvertebrates groups for 28 production days (maintain time) before diminishing. That diminution can be explained by the exhaustion of the production medium in nutritious substances and the damage of organic matter.

The drop of macroinvertebrates density in T_5 the 28th production day corresponds with the duration of the Chironomidae development cycle which varies between 14 and 20 (Madlen, 2005; Kumar and Ramesh, 2012). But the mean duration of the development cycle was in function of several factors of which the main were: the nature and dose of fertilizer, the temperature, the water quality, the species and the sex (Olivera et al., 2003; De Haas et al., 2006, Ozkan and Elipek, 2007; Saliu and Ovuorie, 2007; Epele et al,. 2012; Sulistiyarto et al., 2014).

In addition, it is essential to find simple techniques to maintain the macroinvertebrate maximal density so as to avoid the drop or at best, improve it in order to have them permanently for a long period, for piscicultural purpose. That fall can be avoided in supplying with new doses of dejections periodically in production medium. A part of these organisms population could be periodically harvested for the feeding of fish. It is then after 11 to 12 culture days, the chironomes larvae can be harvested and used as food for the fish *Huso huso* (Sahandi, 2011).

Conclusion

Freshwater benthic macroinvertebrates plurispecific rearing was realizable with pig dung which increased the concentrations production medium in chemical elements like the organism densities. In fact, utilization of different doses of dejections showed that the high doses (T_5, T_6 and T_7) permitted to obtain important macroinvertebrates average productions. But with treatments T_6 and T_7 doses (respectively 75% and 100% of dejection according to substrate volume), we assisted to a brutal drop of population on 21st production day. The treatment T_5 dose (50%

of dejections, that is 150 g of dejections per dm^3 of substrate (150 g/dm^3) constituted the optimal dose of dry pig dung recommendable for benthic macroinvertebrates plurispecifical production in open medium. For, that dose offers conditions for optimal development of organism diverse groups which production was maintained for 28 days.

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