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Suspensions Derived from Anaerobically Fermented Tilapia Offal Inhibit *Fusarium oxysporum* f. sp. *cubense* in Banana

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Abstract

Effective control strategies are lacking for Fusarium wilt of banana crops worldwide. Here, the inhibitory efficacy of suspensions derived from Tilapia offal by anaerobic fermentation against *Fusarium oxysporum* f. sp. *cubense* tropical race 4 (Foc TR4) was evaluated. Two anaerobic fermentation methods were used: 1) natural fermentation of offal (NF) and 2) fermentation of offal supplemented with 5% lime (LF). The suspensions were applied in three treatments: Plate assay, pot system, and in the field. The inhibition rate and disease index were determined. The results showed that the inhibition was significantly greater for LF than for NF on plates. In pot system and in the field, the disease index was lower for the LF group than for the NF group and was significantly lower than that of the control. Therefore, suspensions derived from anaerobically fermented offal provide a new control method for Fusarium wilt in banana.

Keywords

Fusarium Wilt of Banana, Anaerobic Fermentation, Inhibition, Disease Index, Short-Chain Fatty Acid

1. Introduction

Fusarium wilt of banana (*Musa* spp.) is one of the most devastating diseases affecting the banana industry worldwide; however, there are limited effective measures available for managing outbreaks. Despite extensive evaluations of potential control methods based on laboratory, greenhouse, or field experiments, these methods have not yet been applied to actual crops due to certain interactions with complex environmental conditions that lead to unstable effects [1]. In

China, banana plantations are mainly distributed in the Hainan and Guangdong provinces; however, outbreaks of Fusarium wilt have caused a shift to southwestern China as well as to some other ASEAN countries such as Vietnam, Laos, Myanmar, and Cambodia [2]. Without the development of effective disease control strategies, these new plantations may also be at risk of outbreaks of Fusarium wilt in the near future.

A previous study demonstrated that crop straw can be aerobically fermented with antagonistic strains to produce a high-quality fertilizer that prevents or controls plant diseases [3]. Moreover, it was reported that a mixture of cattle waste, vinegar-production residue, and rice straw can be used as a substrate for the solid-state fermentation of *Trichoderma harzianum* SQR-T037, and the resulting bio-organic fertilizer could also effectively control Fusarium wilt in cucumber [4]. Similarly, the bioethanol products from forage rice plants and sweet sorghum fermentation could effectively control the soil-borne pathogen *Fusarium oxysporum* f. sp. *Lycopersici* [5].

Anaerobic fermentation products can also be used to control various diseases in crops. For example, the carboxylic acids released in a barley malt substrate fermented by *Lactobacillus* sp. inhibited the growth of *F. culmorum* macroconidia [6], whey permeate fermented with kefir grains exhibited antifungal effects against *F. graminearum* [7], and anaerobically digested swine manure inhibited the mycelial growth of *F. oxysporum* [8]. These studies highlight the potential of organic acids produced by anaerobic fermentation as anti-fungal agents. In addition, the offal of the freshwater fish tilapia (*Oreochromis mossambicus*) is rich in protein and lipids, and produces numerous short-chain fatty acids such as propionic acid, butyric acid, and valeric acid after anaerobic fermentation [9] [10] [11], which potentially have antibacterial effects. Therefore, we hypothesized that tilapia offal might have inhibitory effects to control Fusarium wilt. To test this hypothesis, in this study, we evaluated the effectiveness of fermentation suspensions derived from anaerobically fermented tilapia offal (with aquatic processing) against *F. oxysporum* f. sp. *cubense* tropical race 4 (Foc TR4) in laboratory, greenhouse, and field experiments, and determined its ability for practical disease control.

2. Materials and Methods

2.1. Suspension Preparation by Anaerobic Fermentation

Tilapia offal was collected from the processing factory of Zhanjiang Guolian Aquatic Products Co. Ltd. Lime was purchased from a building material market in Zhanjiang, China. Suspensions were prepared by subjecting the offal samples (50 kg each) to anaerobic fermentation for 90 d in 50 kg of tapwater [12]. Offal samples were either (1) allowed to naturally anaerobically ferment (NF) or (2) supplemented with 5% lime (M/M) before anaerobic fermentation (LF). The LF treatment was performed because excessive acidic or alkali conditions have been shown to be unsuitable for anaerobic bacteria to effectively decompose the or-

ganic matter, which can be alleviated by the addition of 5% lime (M/M) [13]. Suspension preparations were processed in anaerobic fermenting tanks (100 L; ER-CGER-CG) provided by Wenzhou Yurun Machinery Technology Co., Ltd. (Wenzhou, China)at room temperature.

2.2. Pathogen Sample Preparation

Foc TR4 was provided by the Department of Plant Pathology, South China Agricultural University, Guangzhou, China [14]. The pathogen was maintained on Petri dishes containing potato dextrose agar (PDA) and incubated for 7 d at 28˚C.

2.3. Evaluation of Fermentation Suspensions by Agar Plate Inhibition Assays

In the laboratory, the potential of pathogen inhibition by the offal fermentation suspensions was determined using a visual agar plate assay [15]. A 10% suspension was sterilized and dispensed aseptically into PDA-containing Petri dishes. Mycelial plugs of Foc TR4 were then transferred to the centers of the dishes. Five dishes were used for each suspension treatment (LF and NF), and dishes without any suspension (*i.e.*, plain PDA) were used as the control. The dishes were incubated for 7 d at 28˚C, and then the inhibition rate was calculated according to the following formula [16]:

$$\begin{aligned}&\text{Inhibition rate}(\%)\\&=\left[\left(\text{diameter of the control pathogen colony}-\text{diameter of the test}\right.\right.\\&\left.\left.\text{pathogen colony}\right)/\text{diameter of the control pathogen colony}\right]\times 100\end{aligned}\quad (1)$$

Diameters were determined using a plastic 10-cm ruler.

2.4. Evaluation of Fermentation Suspensions Using a Pot System in a Greenhouse

Pathogen inhibition was evaluated in greenhouse conditions using a pot system as described by Subramaniam *et al.* [17]. Banana cultivars (*Musa acuminata* L. "Fenjiao" AAB) were provided by the Guangdong Academy of Agricultural Sciences, Guangdong, China. The plantlets were individually planted in 16 × 18-cm plastic pots containing sterile soil (2.5 kg) mixed with 100 mL of 10% suspensions (*i.e.*, NF and LF) or sterile water (control). Five independent trials were run sequentially with each trial consisting of nine replicate plants randomly assigned to each treatment. All plantlets were inoculated with 10 mL of a conidial suspension (10^5 conidia mL^{-1}) of Foc TR4 or water (control) near the region between the stem and roots. All pots were watered to saturation and placed in a greenhouse at 28˚C - 32˚C for 3 months. Disease severity was recorded using a scale from 1 to 6 based on the number of discolored leaves as follows: no discolored leaves, grade 1 (value = 0); one discolored leaf, grade 2 (value = 1); two to three discolored leaves, grade 3 (value = 2); four to five discolored leaves, grade 4

(value = 3); five to six discolored leaves, grade 5 (value = 4); and more than six discolored leaves, grade 6 (value = 5). The disease index was then calculated as follows [17]:

$$\begin{aligned}&\text{Disease index}(\%)\\&=\sum\left[\left(\text{number of discolored leaves}\times\text{grade value}\right)/\left(\text{total number}\right.\right.\\&\left.\left.\text{of discolored leaves}\times\text{maximum grade value}\right)\right]\times 100\end{aligned}\quad (2)$$

2.5. Evaluation of Fermentation Suspensions in the Field

The field evaluation was conducted in Xuwen, Zhanjiang, Guangdong Province, China (latitude: 20˚23'; longitude: 110˚30'), which was considered to be a disease hot spot in April–December 2016. The "Fenjiao" plants were transplanted in the field at 2 m × 2-m intervals. Each treatment and control contained 3 blocks of 9 plants each. At the 5 - 6-leaf stage, a 10% suspension or water (control) was sprayed onto the trunk at 2 L per plant. Treatments were applied again at 15 d and 30 d. The disease index was determined at 8 months after planting according to the disease rating scale of 1 - 6, as described above for the greenhouse study. In addition, the disease control rate was calculated as follows:

$$\begin{aligned}&\text{Disease control rate}(\%)\\&=\left[\left(\text{disease index for the control}-\text{disease index for the}\right.\right.\\&\left.\left.\text{treatment}\right)/\text{disease index for the control}\right]\times 100\end{aligned}\quad (3)$$

2.6. Analysis of Short-Chain Fatty Acids in the Fermentation Suspension and Their Antifungal Activity

To determine the underlying inhibitory mechanism, the presence of short-chain fatty acids with reported antimicrobial activity was evaluated. The samples were analyzed by head space-gas chromatography/mass spectrometry by Shanghai Ingeer Certification Assessment Co., Ltd. (Shanghai, China), according to the method described by Araujo *et al.* [18]. The antifungal activity of the main compounds identified was then confirmed using the same pure commercial compound (analytical grade, purchased from Gaolang Chemical Co., Ltd., Shanghai, China). The effects of the commercial compounds on the mycelial growth of Foc TR4 were evaluated by a hyphal extension-inhibition assay [19].

2.7. Statistical Analysis

All statistical analyses were performed using SAS (version 6.12, SAS Institute, Cary, NC) software. Differences were determined by one-way ANOVA, $p < 0.05$ was considered significant. The same software was used to regress probit-transformed inhibition data on the $\log_{10}$-transformed propionic acid and acetic acid concentration. EC50 values for concentrations were obtained from the graph of regression line at the probit zero (50% reduction) point.

3. Results and Discussion

3.1. pH Values of the Fermentation Suspensions

The NF and LF suspensions exhibited different pH values after fermentation of 4.42 and 7.88, respectively. This indicated that although most of the fermented products were acidic, the addition of lime could increase the pH and potentially promote the production of certain substances.

3.2. Fermentation Suspension Evaluation in the Laboratory

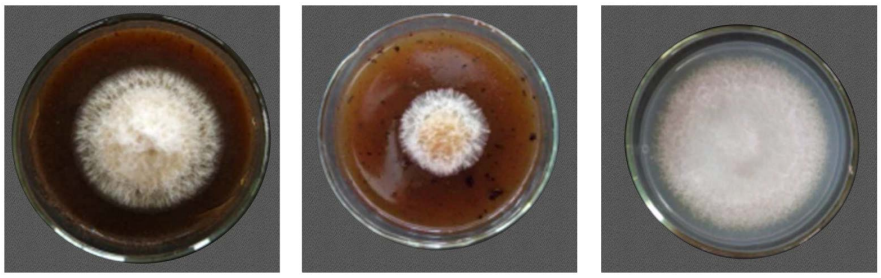
In the laboratory, all fermentation suspensions clearly inhibited pathogen growth. The colonies produced when cultured with the suspensions largely remained at a central location on the plate, whereas the control colonies covered the entire Petri dish in 7 d. However, the rates of inhibition were significantly greater for the LF treatment (87.55%) than for the NF treatment (48.91%, $p < 0.05$; Figure 1).

3.3. Fermentation Suspension Evaluation in the Greenhouse

In the greenhouse, the NF and LF treatments were highly effective in inhibiting the disease when compared to the control in 3 months. The growth of plants with each of the suspensions was normal; the leaves naturally developed and were green, and the pseudo stem sections were colorless. By contrast, the control plants were wilted, the leaves were yellow and dry, and the pseudo stem sections were brown. The average disease index for the LF group was lower (3.70) than that of the NF group (24.44), and all disease indices were significantly lower ($p < 0.05$) than that of the control (94.07; Figure 2).

3.4. Fermentation Suspension Evaluation in the Field

In the field, the NF and LF suspensions were highly effective against the disease compared with the control treatment in 8 months. Similar to the greenhouse experiment, the growth of plants with the suspensions was normal, and the leaves were green, whereas the leaves of the control plants were yellowing and



(a) (b) (c)

Figure 1. Effect of fermentation suspensions on the mycelial growth of Foc TR4 (after 5 d). (a) Offal subjected to natural anaerobic fermentation (NF); (b) offal supplemented with 5% lime before anaerobic fermentation (LF); (c) control [untreated potato dextrose agar (PDA)].

(a) (b) (c)

Figure 2. Effect of fermentation suspensions on Foc TR4 in the greenhouse. (a) Offal subjected to natural anaerobic fermentation (NF); (b) Offal supplemented with 5% lime before anaerobic fermentation (LF); (c) Control.

suspending from the trunk. The disease index for the LF group was lower (2.24) than that of the NF group (7.62), but both suspension groups had a significantly lower disease index ($p < 0.05$) than that of the control (85.16; **Figure 3**). These results indicated that treatment with NF and LF significantly reduced disease severity. In addition, the disease control rate of LF (97.37%) was significantly ($p < 0.05$) higher than that of NF (91.05%).

3.5. Analysis of Short-Chain Fatty Acids in Fermentation Suspensions

In a head space-gas chromatography/mass spectrometry analysis, the main short-chain fatty acids in fermentation suspensions were acetic acid and propionic acid (**Table 1**), both of which were confirmed to have strong inhibitory activity against the growth of Foc TR4. EC50 of propionic acid and acetic acid was 26.24 μg·mL^{-1} and 13.10 μg·mL^{-1}, respectively. Regression equation of propionic acid and acetic acid was y = 2.54 + 1.89x and y = 3.15 + 1.61x, respectively. Correlation coefficient of propionic acid and acetic acid was 0.95276 and 0.95311, respectively (**Figure 4**).

4. Conclusion

Fusarium wilt is one of the most destructive diseases in banana crops worldwide, and only a few management methods have been developed to effectively control outbreaks. Resistant cultivars are considered optimal for managing the disease; however, there are currently limited numbers of cultivars available to support the market demand [20]. In this study, we evaluated the potential of the anaerobic fermentation of tilapia offal, which is rich in protein and lipids, to produce suspensions high in short-chain fatty acids such as propionic acid, butyric acid, and valeric acid [9] [10] for exerting inhibitory effects against *Fusarium* and control disease in banana. Our results from laboratory, greenhouse, and field experiments demonstrated that these suspensions can be safely applied to banana plants to effectively control the development of Foc TR4. Moreover, LF was found to be more effective for inhibiting the pathogen than NF on plates, in the pot system, and in the field. This may be attributed to the effect of lime that not only created an alkaline environment but also increased the yield of fatty acids, thereby improving the anti-fungal effect of the fermentation products. In

(a) (b)

Figure 3. Effect of fermentation suspensions on Foc TR4 in the field. (a) Left as control, right as offal subjected to natural anaerobic fermentation (NF); (b) Left as control, right as offal supplemented with 5% lime before anaerobic fermentation (LF).

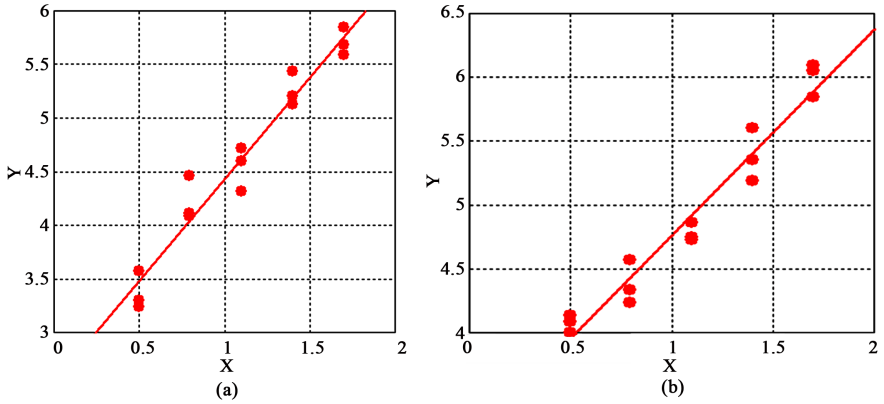


Figure 4. Effects of propionic acid and acetic acid on the mycelial growth of Foc TR4 (after 5 d). (a) Propionic acid; (b) Acetic acid. y = probit-transformed inhibition; x = $\log_{10}$-transformed concentration.

Table 1. The main compounds of short-chain fatty acids of a naturally anaerobically fermented tilapia offal suspension (NF) and tilapia offal suspension with 5% lime added before anaerobic fermentation (LF).

Test item	NF (mg·L^{-1})	LF (mg·L^{-1})
Acetic acid	52.42	57.13
Propionic Acid	10.63	15.99
Isobutyric acid	1.02	5.07
n-Butyric acid	1.18	7.17
Isovaleric acid	1.02	2.01
n-Valeric acid	1.10	2.13

particular, acetic acid and propionic acid were found to be the main components of the suspensions with antifungal activity. Acetic acid is an effective antiseptic and is widely used as an antibacterial agent [21]; however, to our knowledge, there has been no report of the use of acetic acid as an antifungal agent. Since acetic acid achieves an anti-corrosive effect by dissolving the lipids of bacteria cells, the same mechanism could be operating in fungal cells. Propionic acid has been shown to inhibit the growth of mold, although the precise anti-fungal me-

chanism remains unclear [22]. Our results are in agreement with those reported previously [13]. Overall, our study provides new insights regarding the use of suspensions derived from anaerobically fermented tilapia offal against Fusarium wilt in banana. An added advantage of this approach is that it makes full use of waste material, which can not only inhibit the pathogen but also provide organic fertilizer for the soil. Overall, our results can offer a novel strategy for control of the disease, toward improving the health and safety of humans and the environment.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Ploetz, R.C. (2015) Fusarium Wilt of Banana. *Phytopathology*, **105**, 1512-1521. https://doi.org/10.1094/PHYTO-04-15-0101-RVW

[2] Wang, F., Xun, Q.H., Ke, Y.P., Guo, J.C. and Liu, Q.D. (2017) Southeast Asian-China Economic Pattern of Banana Industry Change and Thinking. *Chinese Journal of Tropical Agriculture*, **1**, 12-18. [In Chinese]

[3] Wang, B.B., Yuan, J., Zhang, J., Shen, Z.Z., Zhang, M.X., Li, R., Ruan, Y.Z. and Shen, Q.R. (2013) Effects of Novel Bioorganic Fertilizer Produced by *Bacillus amyloliquefaciens* W19 on Antagonism of Fusarium Wilt of Banana. *Biology and Fertility of Soils*, **49**, 435-446. https://doi.org/10.1007/s00374-012-0739-5

[4] Chen, L.H., Yang, X.M., Raza, W., Luo, J., Zhang, F.G. and Shen, Q.R.M (2010) Solid-State Fermentation of Agro-Industrial Wastes to Produce Bioorganic Fertilizer for the Biocontrol of Fusarium Wilt of Cucumber in Continuously Cropped Soil. *Bioresource Technology*, **102**, 3900-910.

[5] Horita, M. and Kitamoto, H.K. (2015) Biological Soil Disinfestation Using Bioethanol Fermentation Products: Role of Residual Organic Substances. *Journal of General Plant Pathology*, **81**, 304-314. https://doi.org/10.1007/s10327-015-0595-x

[6] Peyer, L.C., Axel, C., Lynch, K.M., Zannini, E., Jacob, F. and Arendt, E.K. (2016) Inhibition of *Fusarium culmorum* by Carboxylic Acids Released from Lactic Acid Bacteria in a Barley Malt Substrate. *Food Control*, **69**, 227-236. https://doi.org/10.1016/j.foodcont.2016.05.010

[7] Gamba, R., De Antoni, G. and Peláez, A. (2016) Whey Permeate Fermented with Kefir Grains Shows Antifungal Effect against *Fusarium graminearum*. *Journal of Dairy Research*, **83**, 249-255. https://doi.org/10.1017/S0022029916000121

[8] Tao, X., Shang, B., Dong, H., Chen, Y. and Xin, H. (2014) Effects of Digestate from Swine Manure Digester on *In Vitro* Growth of Crop Fungal Pathogens: A Laboratory Study. *Transactions of the ASABE* (*American Society of Agricultural and Bio-*

logical Engineers), **57**, 1803-1810.

[9] Işik, O., Sarihan, E., Kuşvuran, E., Gül, Ö.E. and Erbatur, O. (1999) Comparison of the Fatty Acid Composition of the Freshwater Fish Larvae Tilapia zillii, the Rotifer *Brachionus calyciflorus*, and the Microalgae *Scenedesmus abundans*, *Monoraphidium minitum* and *Chlorella vulgaris* in the Algae-Rotifer-Fish Larvae Food Chains. *Aquaculture*, **174**, 299-311. https://doi.org/10.1016/S0044-8486(99)00013-7

[10] Yuan, H.Y., Chen, Y.G., Zhang, H.X., Jiang, S., Zhou, Q. and Gu, G.W. (2006) Improved bioproduction of short-chain fatty acids (SCFAs) from excess sludge under alkaline conditions. *Environmental Science & Technology*, **40**, 2025-2029. [In Chinese]. https://doi.org/10.1021/es052252b

[11] Li, L., He, G., Xin, X.D., Wang, M.F., Xu, J. and Zhang, J. (2017) Enhanced Bioproduction of Short-Chain Fatty Acids from Waste Activated Sludge by Potassium Ferrate Pretreatment. *Chemical Engineering Journal*, **332**, 456-463. https://doi.org/10.1016/j.cej.2017.09.103

[12] Han, S.K. and Shin, H.S. (2004) Biohydrogen Production by Anaerobic Fermentation of Food Waste. *International Journal of Hydrogen Energy*, **29**, 569-577. https://doi.org/10.1016/j.ijhydene.2003.09.001

[13] Su, G.Q., Huo, M.X., Yuan, Z.G., Wang, S.Y. and Peng, Y.Z. (2013) Hydrolysis, Acidification and Dewaterability of Waste Activated Sludge under Alkaline Conditions: Combined Effects of NaOH and $Ca(OH)_2$. *Bioresource Technology*, **136**, 237-243. https://doi.org/10.1016/j.biortech.2013.03.024

[14] Li, M.H., Shi, J.X., Xie, X.L., Leng, Y.Q., Wang, H.F., Xi, P.G., Zhou, J.N., Zhong, S.B. and Jiang, Z.D. (2013) Identification Ad Application of a Unique Genetic Locus in Diagnosis of *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4. *Canadian Journal of Plant Pathology*, **35**, 482-493. https://doi.org/10.1080/07060661.2013.828321

[15] Nel, B., Steinberg, C., Labuschagne, N. and Viljoen, A. (2007) Evaluation of Fungicides and Sterilants for Potential Application in the Management of Fusarium Wilt of Banana. *Crop Protection*, **26**, 697-705. https://doi.org/10.1016/j.cropro.2006.06.008

[16] Roberts, W.K. and Selitrennikoff, C.P. (1986) Isolation and Partial Characterization of Two Antifungal Proteins from Barley. *BBA Biomembranes*, **880**, 161-170.

[17] Subramaniam, S., Maziah, M., Sariah, M., Puad, M.P. and Xavier, R. (2006) Bioassay Method for Testing Fusarium Wilt Disease Tolerance in Transgenic Banana. *Scientia Horticulturae*, **108**, 378-389. https://doi.org/10.1016/j.scienta.2006.02.028

[18] Araujo, P., Nguyen, T.T., Froyland, L., Wang, J.D. and Kang, J.X. (2008) Evaluation of a Rapid Method for the Quantitative Analysis of Fatty Acids in Various Matrices. *Journal of Romatography A*, **1212**, 106-113.

[19] Allen, T.W., Enebak, S.A. and Carey, W.A. (2004) Evaluation of Fungicides for Control of Species of Fusarium on Long Leaf Pine Seed. *Crop Protection*, **23**, 979-982. https://doi.org/10.1016/j.cropro.2004.02.010

[20] Ploetz, R.C. (2015) Management of Fusarium Wilt of Banana: A Review with Special Reference to Tropical Race 4. *Crop Protection*, **73**, 7-15. https://doi.org/10.1016/j.cropro.2015.01.007

[21] Huo, Z., Fang, Y., Yao, G., Zeng, X., Ren, D. and Jin, F. (2015) Improved Two-Step Hydrothermal Process for Acetic Acid Production from Carbohydrate Biomass. *Journal of Energy Chemistry*, **24**, 207-212. https://doi.org/10.1016/S2095-4956(15)60302-3

[22] Chen, L., Yuan, X.J., Li, J.F., Wang, S.R., Dong, Z.H. and Shao, T. (2017) Effect of Lactic Acid Bacteria and Propionic Acid on Conservation Characteristics, Aerobic Stability and *in Vitro* Gas Production Kinetics and Digestibility of Whole-Crop Corn Based Total Mixed Ration Silage. *Journal of Integrative Agriculture*, **16**, 1592-1600. https://doi.org/10.1016/S2095-3119(16)61482-X