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Anthracnose photo by UM Amherst, USA

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Smith J, Jones M Jr, Houghton L et al. 1999. Future of health insurance. N Engl J Med 965: 325-329. DOI: 10.10007/s002149800025.

Book:

Rai MK, Carpinella C. 2006. Naturally Occurring Bioactive Compounds. Elsevier, Amsterdam.

Chapter in the book:

Webb CO, Cannon CH, Davies SJ. 2008. Ecological organization, biogeography, and the phylogenetic structure of rainforest tree communities. In: Carson W, Schnitzer S (eds.). Tropical Forest Community Ecology. Wiley-Blackwell, New York.

Abstract:

Assaeed AM. 2007. Seed production and dispersal of *Rhazya stricta*. 50th annual symposium of the International Association for Vegetation Science, Swansea, UK, 23-27 July 2007.

Proceeding:

Alikodra HS. 2000. Biodiversity for development of local autonomous government. In: Setyawan AD, Sutarno (eds.). Toward Mount Lawu National Park; Proceeding of National Seminary and Workshop on Biodiversity Conservation to Protect and Save Germplasm in Java Island. Universitas Sebelas Maret, Surakarta, 17-20 July 2000. [Indonesian]

Thesis, Dissertation:

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Short Communication: Response of insect foragers to four soybean mutant lines cultivated in savanna agro-ecology of Ghana

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Abstract. *Philip AA, Adu-Acheampong S, Addai IK. 2020. Short Communication: Response of insect foragers to four soybean mutant lines cultivated in savanna agroecology of Ghana. Asian J Agric 5: 1-3.* To increase soybean production, different lines have been developed with improved qualities to withstand problems associated with the production process. However, such development can render the lines (e.g., mutant lines) susceptible to pest attacks. We sampled for insect abundance and diversity within four soybean lines (150Gy, 200Gy, 250Gy, 300Gy) cultivated under two levels of NPK-15-15-15 fertilizer to check if mutagenesis has increased their attraction to insect activities including pest attacks. Some of the species recorded were *Acanthacris ruficornis*, *Apis mellifera*, *Spodoptera frugiperda*, *Helicoverpa armigera*, *Bemisia tabaci*, and *Callosobruchus* spp. The study also realized that NPK-15-15-15 had no significant impact on insects recorded within the various mutant lines. This only translates into no special pest attraction due to mutagenesis of not for any line. We recommend from this study that farmers should not have any reservation for cultivating any recommended mutant line for mass production after all other tests are completed on them before release.

Keywords: Fertilizer, foragers, insect, lines, mutagenesis, soybean

INTRODUCTION

To increase soybean production especially in northern Ghana, different lines and varieties have been developed (Santos 2019) (e.g., through metagenesis) with improved qualities to withstand problems associated with production such as pod shuttering, moisture stress, and disease susceptibility (Maganoba 2018). However, breeding against these traits can potentially predispose these lines to other unforeseen problems. For instance, several studies on mutagenesis of leguminous crops show that some properties of the crop can be altered after the change (Shekar 2017; Maganoba 2018; Singh and Sadhukhan 2019). Such changes may either be positive to bring more desirable qualities or negative and ranging from changing days to maturity, water retention or stress tolerance, pod shattering ability and even increasing susceptibility to pest attacks (Shekar 2017; Maganoba 2018; Singh and Sadhukhan 2019). As a result, developing improved varieties of the crop must factor in the susceptibility of the crop pest due to unforeseen losses it can impose on production. Although, these lines (150Gy, 200Gy, 250Gy, 300Gy) have been developed they are yet to be evaluated for their attraction or otherwise to insect foragers that may also include pests.

In accordance with that, we set out a field experiment to investigate the attractiveness or otherwise, of these four soybean lines developed through mutagenesis to insect foragers which also included pests as a measure of plant vigor. We hypothesized that mutagenesis has not rendered these lines more attractive to insect foragers that include pests. If so, we expect no significant differences in diversity and abundance of insect foragers between the different mutant lines. We specifically quantified the difference in abundance and diversity of insect foragers associated with the different mutant lines and determined the impact of fertilizers on the abundance of insect foragers (if any) within each of the mutant lines as a measure of their vigor under fertilization.

MATERIALS AND METHODS

Study site

The research was carried out at the University for Development Studies (UDS) Experimental Farm, Nyankpala, Ghana during the 2018 cropping season. Nyankpala is in the Tolon District, about 20 km southwest of Tamale in northern Ghana. It has a latitude of 9° 25'41"N and longitude of 0° 58' 42" W with an altitude 200 m above sea level (Serno and van der Weg 1985). Nyankpala is situated in the Guinea savanna agroecology with a tropical climate. Nyankpala experiences a unimodal pattern of rainfall with mean annual rainfall of 118.61 mm within 95 days with average annual temperature of 28.2°C (Nkrumah et al. 2014).

Field layout and experimental materials

The experimental field was demarcated into 60 plots of dimensions 4m x 3m with 3 replications in a Randomized Complete Block Design and a 1 meter spacing between blocks and plots. Four soybeans mutant lines (150Gy, 200Gy, 250Gy, 300Gy) were used for the experiment. The mutant lines were developed from a local variety 0 Gy which was used as a control. These mutant lines have been under evaluation since 2016 (Maganoba et al. 2018). We obtained these seeds from the Department of Agronomy, University for Development Studies, Nyamkpala-Tamale, Ghana.

Soybean cultivation and insect sampling

Planting was done at two (2) seeds per hole with a first manual weeding at two (2) weeks and the second at six (6) weeks after germination. Fertilizer was applied to plots marked for NPK-15-15-15 six (6) weeks after planting at a rate of 200 kg.ha^{-1}. Visual observation, handpicking and sweep net sampling were the main methods used for the study. Insect collections were transferred into the entomology laboratory of UDS and identified to the highest possible taxon. Sampling was done on four occasions at two-week intervals.

RESULTS AND DISCUSSION

Abundance of insect foragers

The study recorded a variety of species of insect foragers of soybean including pod, foliage, and flower feeders. Some of the recorded species were the fall armyworm, grasshoppers, bees, beetles, and bugs with both beneficial and pestiferous species. This record of occurrence of both beneficial insects and pestiferous ones (see table 1 below) agrees with a similar study by Hartman et al. (2016), which stated that there are both beneficial and harmful insect species associated with soybean.

Soybean lines and insect abundance

The study results further show that the control group (0Gy) recorded the highest insect abundance of 357 individuals followed by 300Gy (327) while 200Gy and 250Gy recorded 306 and 308 individuals respectively with 150Gy recording the least abundance of 301 individuals, but these differences were not statistically significant ($P = 0.93$). Also, there was no significant difference in species diversity between the different lines ($P > 0.05$).

The abundance and diversity of insect foragers recorded within these lines show that mutagenesis of the control line (0Gy) did not affect its attractiveness to insect foragers which also include pests as reported in other studies elsewhere (Narusaka et al. 2003; Scholz et al. 2014).

Impact of fertilizer on insect foragers abundance

Mutant lines that received 15-15-15 NPK fertilizer recorded the least number of insect foragers (762) individuals, while those that did not receive fertilizer recorded the highest abundance of 837. However, there was no statistically significant difference in both abundance and diversity between the lines with or without fertilizer ($P = 0.23$). This finding agrees with other studies that reported that fertilizers rarely affect arthropods directly although it influences the other characteristics of the plant such as, biochemistry morphology, and physiology which can indirectly affect the ecology of insects because of changes in dynamics of available food (Shikano 2017; War et al. 2018; Han et al. 2019).

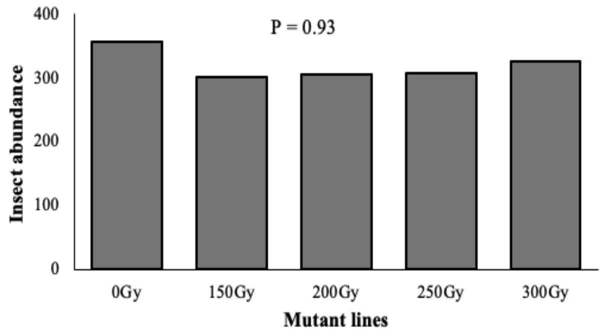

Figure 1. Response of soybean mutant lines to insect foragers abundance in Nyankpala, Ghana

Table 1: The list of some common insect foragers identified in soybean field at the UDS-Experimental Field at Nyankpala, Ghana

Common name	Scientific name	Family
Southern green stinkbug	*Nezara viridula*	Pentatomidae
Green semilooper	*Thysanoplusia orichalcea*	Noctuidae
Giant grasshopper	*Valanga* spp.	Acrididae
Black leaf beetle	*Plagiodera* spp.	Chrysomelidae
Spined soldier bug	*Podisus* spp.	Pentatomidae
Bruchid beetles	*Callosobruchus spp*	Chrysomelidae
Honeybee	*Apis mellifera*	Apidae
Flea beetle	*Disonycha* spp.	Cydnidae
Bean pod borer	*Maruca vitrata*	Erebidae
Cotton bollworm	*Helicoverpa armigera*	Chrysomelidae
The garden locust	*Acanthacris ruficornis*	Acrididae

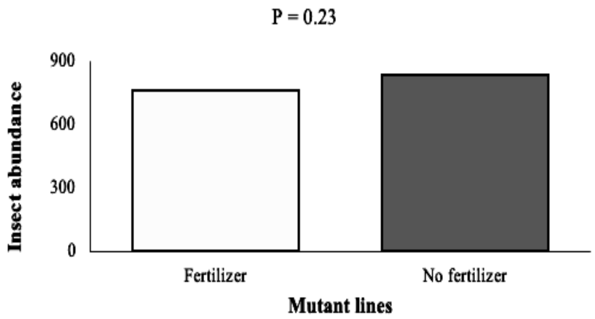

Figure 2. Impact of fertilizer on insect foragers abundance in Nyankpala, Ghana

We recommend that farmers should not have any reservations about using any recommended mutant line after screening for production because none of the lines have shown any special attraction to insect foragers which also includes pests.

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Evaluation of some pesticides of plant origin for control of anthracnose disease (*Colletotrichum destructivum* O'Gara) in cowpea

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Manuscript received: 29 July 2020. Revision accepted: 12 October 2020.

Abstract. *Enyiukwu DN, Amadioha AC, Ononuju CC. 2021. Evaluation of some pesticides of plant origin for control of anthracnose disease (*Colletotrichum destructivum *O'Gara) in cowpea. Asian J Agric 5: 4-11.* Anthracnose is a common disease of cowpea in many bean growing areas of the world. This study evaluated the effects of *Alchornea cordifolia, Tabernaemontana pachysiphon*, and *Lantana camara* as low-input biopesticides for control of the disease. The experiment was laid out in randomized complete block design (RCBD) made up of 14 treatments with 4 replications. The results indicated that all the plant materials irrespective of carrier solvent and concentrations of application significantly ($P \leq 0.05$) minimized the incidence and severity of the disease, as well as improved the yield and yield parameters of the treated crop than the control. Amongst all evaluated dosages of the plant materials, 50-100 % concentration of *L. camara* gave the best disease control and yield improvement of the crop, followed by full strength of *T. pachysiphon* and *A. cordifolia* was the least. However, comparative to benomyl a standard fungicide, the plant-derived pesticides demonstrated lower fungitoxicity against the pathogen apart from 50-100 % extracts of *L. camara* which were statistically ($P \geq 0.05$) at par with the effects of the fungicide. Therefore, all the plant extracts could be used at higher doses as prophylactics to stem the disease; however, *L. camara* could be applied at lower doses to achieve the same level of control. These plant materials overall could therefore contribute as effective bio-fungicides towards improving productivity of cowpea in the humid tropics.

Keywords: Anthracnose, bio-pesticides, cowpea, fungitoxicity, plant disease, plant extracts

INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp.), otherwise called southern pea, is an important legume in tropical Africa and some parts of Asia where it is widely consumed as leafy vegetable and grain legume (Nielsen et al. 1997; Enyiukwu et al. 2018a). Anthracnose caused by *Colletotrichum destructivum* O'Gara has been identified as one of the major pathogen-induced challenges affecting its production in the humid tropics (Adegbite and Amusa 2008; Enyiukwu et al. 2014). Owing to the many drawbacks associated with use of synthetic pesticides in agriculture and public health, alternatives from natural sources especially higher tropical plant species are being vigorously sought (Awurum and Enyiukwu 2013; Awurum et al. 2016).

Alchornea cordifolia (Euphorbiaceae) is a staggering evergreen small tree that occurs sporadically in the rain forest and savannas of the tropics. On the other hand, *Tabernaemontana pachysiphon* (Apocynaecea) is a tropical evergreen tree used occasionally as ornamental plant, while *Lantana camara* (Verbenaceae) is a prickly invasive shrub found in many countries of Africa and Australia (Enyiukwu 2017). These plants have a long history of usage as phytotherapeutic agents against several human bacterial and fungal pathogens such as: *Klebsiella sp., Staphylococcus sp. Proteus sp.* and *Candida sp.* (Okwu and Ukanwa 2010; Enyiukwu 2017). Recently methanol extracts of these medicinal plant species demonstrated considerable fungi toxic activity against *C. destructivum* O'Gara, a destructive plant pathogen responsible for anthracnose disease of some legumes *in vitro* (Enyiukwu 2017).

Therefore, this work aimed at evaluating the effects of aqueous and methanol extracts of *L. camara., T. pachysiphon,* and *A. cordifolia* against the initiation, development, and spread of anthracnose disease caused by *C. destructivum* O'Gara in cowpea and performance of the treated crop in the screen-house.

MATERIALS AND METHODS

Experimental site and location

The experiment was conducted at the Plant Health Management Laboratory and Greenhouse of the College of Crop and Soil Sciences of Michael Okpara University of Agriculture, Umudike (MOUAU), Nigeria in August to October, during the 2015 cropping season. The environmental parameters of the location during the study months were: rainfall between 276-280.20 mm, a temperature range of 25-30°C, and relative humidity from 74-87.0 %. The soil type was sandy clay with organic carbon recorded at 68000 ppm, while organic matter was 10300 ppm, pH 4.17; phosphorus 26.55 mg/kg and calcium 2.10 Cmol/kg. The location stands at an altitude of 121.08 meters above sea level (GPS Coordinates 2017).

Preparation of extracts of the plant materials

Leaves of *A. cordifolia T. pachysiphon* and *L. camara* were washed under running tap and rinsed in 500 ml of sterile distilled water, air-dried on the laboratory bench for 3 weeks, and then using a hand milling machine they were separately milled into fine powder (300 g of each specimen) and stored separately in air-tight bottles. About 25, 50, 75, and 100 g of each powdered specimen were weighted out and soaked separately in 100 ml of sterile distilled water or same quantity of 30 % methanol contained in 250 ml conical flasks and allowed to stand for 6 h. Thereafter they were sieved separately through 4-folds of cheesecloth into different 200 ml sterile beakers to obtain the filtrates of 25, 50, 75, and 100 % concentrations of the aqueous and methanol extracts respectively (Amadioha 2003).

Isolation, identification, and preparation of spore suspension of the causal agent

Culture medium was prepared using dehydrated potato dextrose (PDA) (Oxoid™ ThermoScientific Product, England, UK), while the pathogen was isolated from infected cowpea, subjected to pathogenicity tests, and identified. The spores suspension of the isolated pathogen was subsequently prepared and standardized using a hemocytometer counting slide to 1.0×10^5 spores/ml of sterile distilled water (generally reported in literature as the concentration of pathogenic fungal spores in experiments; that can effectively initiate infection in susceptible host plants) (Amadioha 2003; Alberto 2013; Nwaoguikpe et al. 2014).

Evaluation of the effects of the extracts of the plant materials on infected cowpea in the screen-house

Four weeks after sprouting, healthy cowpea (Var. IAR-48) seedlings growing in pots containing heat-sterilized topsoil (4 kg) were spray-inoculated to run-off with spore suspension of *C. destructivum* (1×10^5 spores/ml), and subsequently grouped into two groups A and B; which were simultaneously treated with the test bio-pesticides. Seedlings in Group A were sprayed with aqueous suspensions of the different concentrations (25, 50, 75, and 100%) of the plant extracts, while those in Group B were sprayed with corresponding concentrations of methanol extracts of the plant materials. The control experiments were set up in a similar manner but sprayed only with suspension of the fungus and sterile distilled water or benomyl. The experiment was arranged in a randomized complete block design (RCBD) made up of 14 treatments and 4 replicates. The incidence and severity of the disease on the inoculated cowpea seedlings were assessed according to the formula adopted by Amadioha (2003) as follows:

$$\% \text{ Incidence} = \frac{\text{Number of diseased plants}}{\text{Total number of plants examined}} \times 100$$

The disease severity was assessed by visual assessment of the test plants with typical symptoms of the anthracnose disease, using the descriptive scale of 1-10 as outlined by Enyiukwu and Awurum (2013b):

Where :

1. no disease;
2. 1-25% of seedling with anthracnose disease;
4. 26-50% of seedling with anthracnose disease;
6. 51-75% of seedling with anthracnose disease;
8. 76-100% of seedling with anthracnose disease and
10. Stem breakage, girdling or death of seedling due to anthracnose disease.

The fungitoxicity of the extracts was evaluated as reduction in severity of anthracnose disease on the potted seedlings sprayed with spore suspension and the extracts of varying concentrations compared to the control experiments treated with water or benomyl alone (Amadioha 2003).

Evaluation of effects of extracts of the plant materials on height, number of leaves, and biological yield of cowpea

The growth parameters (height and number of leaves) of the two groups of the inoculated potted cowpea plants after exposure to the various concentrations of the test plant extracts were recorded at weeks 6 and 8 after planting (WAP). However, the effects of the treatments on the biomass accumulation of each treated plant were recorded only at 8 WAP. At this time, the treated cowpea was harvested (uprooted) and oven-dried at 60ºC for 3 days and the weight of the dry matter content of the treated plants was measured using a digital balance and recorded according to the protocols adopted by Awurum and Ogbonna (2013) and Awurum et al. (2016).

RESULTS AND DISCUSSION

Effects of the extracts on the incidence and severity of anthracnose disease of cowpea in the screen-house

Results of Table 1 indicated that high mean incidence (83%) and severity (approx. 6) of anthracnose disease were recorded on the untreated cowpea (control experiment) compared to nearly 19.00 % and 1.45; 20.40 % and 1.80; 27.00 % and 2.06; and 30.00 % and 2.22 recorded for incidence and severity of the disease on benomyl, *L. camara, T. pachysiphon,* and *A. cordifolia* treated cowpea respectively. The result of the effects of the concentrations of the different botanicals revealed that the crude extracts of the test plants significantly inhibited the initiation, development, and spread of the fungus in cowpea (Table 1). The 100 % concentration of the *L. camara* reduced the incidence of *C. destructivum* induced anthracnose disease in the test cowpea from 55 % and 85 % recorded on the control at 6 WAP and 8 WAP to 10 % and 20 % for the methanol and 10 % and 22 % for water extract respectively. Also, the disease severity indices were reduced from 4.1 and 5.71 recorded on the control experiment to 1.0 and 2.02 for methanol extract (100 %) and 1.0 and 1.90 for water extract of *L. camara* after 6 and 8 WAP. Both methanol and water extracts of *T. pachysiphon* and *A. cordifolia* at 100 % concentration recorded lower disease incidence and severity when compared with the control (Table 1). There was no significant difference between the results recorded with benomyl (benzimidazole) and 75-100% concentration of *L.*

camara extracts in terms of disease severity. There also was no significant difference in fungitoxicity of benomyl when compared with methanol and water extracts of the test plants in disease incidence on cowpea both at 6 WAP and 8 WAP (Table 1).

The toxicity profile of the botanicals was *L. camara*>*T. pachysiphon*>*A. cordifolia*. The results also revealed that irrespective of the type of plant material evaluated, the fungitoxicity increased with the concentration of the test plant extracts. The fungi toxic activity of the aqueous extracts was not statistically ($P<0.05$) superior to methanol extracts of the plant materials against the target fungus.

The mean effects of the different levels of the bio-pesticides on the disease progression of the *C. destructivum*-inoculated cowpea (Figure 1 and 2) (see Table 3) indicated that the disease progressed very rapidly, increasing exponentially between 4 and 6 WAP hence the sharpest slope and largest area under the growth curve within this period. However, it increased at a decreasing rate from 6-8 WAP. This suggests that the disease caused by *C. destructivum* was severe within two weeks (6 WAP) having severity index of 1.6 times greater than infection rate at 4 WAP.

Effects of the plant extract on the growth parameters and biological yield of cowpea

The results presented in Table 2 indicated that the plant extracts had significant effects on the growth and biological yield of cowpea. Amongst the plant extracts, the best dry matter yield of 4.30 g, 4.41 g, and 4.52 g were obtained from *L. camara* treated cowpea at 50 %, 75%, and 100% concentrations respectively. There was no significant difference in the dry matter yield (4.62 g) obtained from benomyl (benzimidazole) and those recorded with 50-100% concentrations of the methanol extracts of *L. camara*. Also, there was no significant difference with benomyl (4.49 g) when compared with respective yields of 4.19 g, 4.38 g, and 4.41 g from 50, 75, and 100% concentrations of the water extracts of the plant materials on the test cowpea 8 WAP (Table 2). However, the dry matter yields obtained from all the extracts of the test plants irrespective of concentration, and solvent of extraction were statistically ($p\leq0.05$) greater than those recorded for the control experiment.

All the extracts irrespective of concentration and type of plant material significantly ($P\leq0.05$) enhanced the plant height and number of leaves as well as dry matter yield of the crop over the control. In terms of number of leaves, except for *T. pachysiphon* at 50% concentration, all the assayed plant extracts irrespective of concentration and carrier solvent significantly ($P\leq0.05$) increased the number of leaves of the test crop over the control 8 WAP and compared favorably ($P\leq0.05$) also with those recorded for benomyl treated cowpea at both 6 WAP and 8 WAP. Similarly, all the plant extracts tested at 100 % concentration had a better performance than the control experiment in improving the height of the cowpea at 6 WAP and 8 WAP except *A. cordifolia*. In general, the best yield and yield attributes were recorded when the crop was treated with *L. camara* followed by *T. pachysiphon.* However, in terms of dry matter yield 50%, 75%, and 100% concentrations of methanol and water extracts of *L. camara* was statistically ($P\leq0.05$) at par with result obtained from benomyl treatment.

Table 1. Effects of the aqueous and methanol extracts of test plants and benomyl against *Colletotrichum destructivum* in cowpea eight weeks after planting (8 WAP)

Treatment	Disease incidence (%), severity, and time of planting (weeks)							
	6 WAP		8 WAP		6 WAP		8 WAP	
	DI	DS	DI	DS	DI	DS	DI	DS
Alchornea cordifolia								
25%	30	2.0	41	3.23	28	2.0	35	2.20
50%	30	2.0	40	2.84	27	2.0	32	2.20
75%	24	2.0	33	2.45	22	2.0	31	2.17
100%	22	2.0	32	2.41	22	2.0	30	2.08
Tabernaemontana pachysiphon								
25%	25	2.0	35	2.31	23	2.0	30	2.11
50%	23	2.0	33	2.30	21	2.0	30	2.01
75%	23	2.0	32	2.40	20	2.0	29	2.01
100%	20	1.8	30	2.22	20	2.0	27	2.00
Lantana camara								
25%	20	2.0	28	2.18	20	2.0	30	2.10
50%	18	2.0	26	2.05	18	2.0	24	2.05
75%	15	1.5	23	2.03	16	1.0	26	1.95
100%	10	1.0	20	2.01	10	1.0	22	1.90
Benomyl (3g/l)	11	1.2	17	1.69	10	1.0	20	1.91
Sterile water	55	4.1	85	5.71	51	4.0	82.9	5.64
LSD (0.05)	**7.3**	**1.1**	**4.2**	**0.78**	**1.9**	**0.9**	**5.02**	**0.57**

Note: Values are means of 4 replicates in two separate experiments; DI: disease incidence (%); DS: disease severity

Table 2. Effects of aqueous and methanol extracts of test plants and benomyl on height, number of leaves, and yield of cowpea 6 and 8 WAP

Treatment	Agronomic parameters and time of planting (weeks)									
	6 WAP		8 WAP			6 WAP		8 WAP		
	PH	NL	PH	NL	DM	PH	NL	PH	NL	DM
Alchornea cordifolia										
25%	23	3.01	32	4.04	3.18	22	3.00	30	4.11	3.17
50%	23	3.02	33	4.08	3.24	23	3.05	31	4.14	3.21
75%	27	3.40	37	4.34	3.38	25	3.12	32	4.17	3.49
100%	28	3.40	41	4.41	3.73	30	3.21	35	4.28	3.61
Tabernaemontana pachysiphon										
25%	25	3.00	32	4.22	3.90	24	3.20	33	4.21	3.58
50%	23	3.00	34	3.30	3.90	25	3.20	33	4.22	3.68
75%	24	3.20	33	4.31	4.09	25	3.31	38	4.30	3.78
100%	23	3.40	33	4.40	4.10	27	3.40	39	4.30	3.97
Lantana camara										
25%	23	3.20	36	4.05	4.10	23	3.10	34	4.15	4.12
50%	24	3.41	36	4.18	4.30	23	3.23	34	4.33	4.18
75%	24	3.50	38	4.43	4.41	24	3.28	36	4.38	4.29
100%	26	3.51	40	4.61	4.53	25	3.40	42	4.95	4.38
Benomyl (3g/l)	26	3.45	41	4.57	4.62	25	3.29	38	4.81	4.49
Sterile water	23	2.38	29	3.16	2.05	21	2.48	29	2.64	2.18
LSD (0.05)	**2.2**	**0.25**	**6.02**	**0.78**	**0.38**	**1.92**	**0.9**	**5.1**	**0.57**	**0.33**

Note:PH: Plant height (cm); NL: Number of leaves; DM: Dry matter content of biological yield of treated whole cowpea plant (grams)

Table 3. Mean effects of different concentrations of water and methanol extracts of the plant materials on anthracnose incidence in cowpea

Plant materials	Extracting solvent and weeks after planting (WAP)				
	Week 4	Week	6	Week	8
	Both solvents	Water	Methanol	Water	Methanol
Alchornea cordifolia	0	25.00	26.50	32.00	36.50
Tabernaemontana pachysiphon	0	23.30	2280	30,50	32.50
T. camara	0	16.00	15.80	25.00	24.30
Benomyl	0	10.00	11.00	20.00	17.00
Distilled water	0	51.00	55.00	82.90	85.00
Total	0.00	125.00	131.10	190.40	195.30
Mean	0	25.00	26.22	38.08	39.06

Discussion

The results of Table 1 showed that high mean incidence and severity of anthracnose disease were recorded on the untreated control experiment. Mohammed (2013) reported that high ambient temperature, rainfall, and relative humidity encourage the initiation, development, and spread of anthracnose disease of cowpea. The high mean incidence (83%) and severity (approx. 6) recorded on the untreated control experiment in this study may have been favored by high ambient temperature (29-31ºC), rainfall (276-280.2 mm), and relative humidity (74-87%) which lasted for up to 23 days in the study months (September-October 2015). Well-drained, slightly acidic, sandy clay soil with a pH 5, that was rich in organic matter, organic carbon, and low in salt (sodium) content permits optimal growth and performance of cowpea (Anyanwu et al. 1980; Timko et al. 2007). Similarly, low calcium and phosphorus contribute to poor development of resistance mechanisms in crops (Owolade et al. 2006; King et al. 2012; Boumaaza et al. 2015). Hence, the poor phosphorus (26.55 mg/kg) and calcium (2.10 Cmol/kg) status of the greenhouse soil may have informed the poor resistance of the test crop to *C. destructivum* resulting in the high mean incidence and severity of anthracnose disease recorded on the control experiment in this study.

Table 1 also indicated that irrespective of type of carrier solvent and concentration of application of the botanicals used in this study, all the test extracts performed well in minimizing the incidence and severity of anthracnose disease of cowpea in the field which was statistically greater (P≤0.05) than the control. However, benomyl demonstrated a superior fungitoxicity over the botanicals except for 50-75% concentration of *L. camara*. This implies that all of the test plants could be used as prophylactics against anthracnose disease of cowpea since they minimized the development and expression of the disease at 4 weeks after planting in the field.

Several workers have demonstrated the efficacy of pesticides of plant origin against several pathogenic fungi in the field. Field trials in Nigeria and Brazil showed that extracts of *Ocimum sanctum*, *Piper nigrum*, *Vernonia polyanthus Xylopia aethiopica*, *Cymbopogon citratus,* and *C. flexuosus* seriously affected the development and spread of anthracnose caused by *C. lindemuthianum* on cowpea (Amadioha 2001; 2003; Lemos da Silva et al. 2015). Similarly, *P. guineense* seed extract was reported to effectively minimize *C. destructivum*-induced anthracnose lesions on treated cowpea in the field (Enyiukwu and Awurum 2013). The effectiveness of these botanicals was found in most evaluations to compare well with the effect of the synthetic fungicides such as benomyl, thiophanate-methyl or Celest® in a dose-dependent manner (Enyiukwu and Awurum 2013; Masangwa et al. 2013). The findings in this study where the extracts of *L. camara, T. pachysiphon*, and *A. cordifolia* reduced the incidence and severity of anthracnose disease of cowpea in the field in a dose-dependent manner are in agreement with the earlier reports by other scientists as mentioned above.

Botanicals are reported to contain several bioactive chemical groupings such as terpenoids, alkaloids, saponins, steroids, flavonoids, tannins, glycosides, and fatty acids; these have been reported to underpin the bioactivity of these plant materials (Edeoga et al. 2005; Enyiukwu and Awurum 2013a). Presence of alkaloids, flavonoids, saponins, tannins, polyphenols, glycosides, and terpenoids have been reported in *L. camara, T. pachysiphon,* and *A. cordifolia* used in this study (Duru and Mbata 2010; Pradhan et al. 2012; Nwaoguikpe et al. 2014). Fatty acids including 9,12-Octadecadienoic acid methyl ester, 9-Octadecenoic acid methyl ester, and Dodecanoic acid (1,2,3-propanetryl ester) isolated from these plant materials (Enyiukwu 2017) are known to be antimicrobial compounds (Okwu and Njoku 2009; Akpuaka et al. 2013); suggesting that the fungitoxicity of the plant materials used against the test pathogen in this investigation may be due to these compounds. The high presence of these fatty acids, especially dodecanoic (lauric) acid in *L. camara* followed by *T. pachysiphon* , may have informed their higher antifungal activity over *A. cordifolia* on *C. destructivum* O'Gara in this study (Enyiukwu 2017).

Earlier field evaluations from Amadioha and Obi (1998; 1999) reported the superiority of fungi toxic activity of extracts of *Xylopia aethiopica* and *Azadirachta indica* over benomyl in minimizing *Colletotrichum lindemuthianium* incited anthracnose lesions in cowpea. Awurum and Ucheagwu (2013) also found *Piper guineense* in a similar manner to out-perform benomyl in protecting cowpea seeds in storage against *Fusarium* and *Colletotrichum* induced deteriorations over a 3-month period. Findings from this study, however, did not agree with these submissions since the extracts of the test plants used in this assay were not as effective as benomyl in arresting the initiation, development and spread of the pathogenic fungus (Table 1). The development of resistance of *Colletotrichum spp.* to benomyl (Tu 1981) and tolerance to its close chemical relatives carbendazim and thiophanate-methyl (Emechebe and Florini 1997) have been reported and this may explain the lower sensitivity of the pathogen to the fungicide as reported by the other investigators.

The disease progress curves (Figures 1 and 2) suggest that the control strategies against cowpea anthracnose disease should be preventive and such measures must be initiated early in the cycle (4 WAP) to stem the disease. High incidence and severity of *Colletotrichum spp.* induced anthracnose, brown blotch, and related fungal diseases following natural or artificial infection of seedling of *Allium cepa* and cowpea at 2-4 WAI have been reported by Owolade et al. (2004) and Awurum et al. (2016) respectively. These investigators maintained that the diseases were very severe during this period, progressing rapidly and extensively 2-3 weeks post-infection with the pathogens. Data from this study affirm that the attacks and severity of *C. destructivum* induced anthracnose disease in the test cowpea progressed rapidly and severely at this same period on the test seedlings. Extracts of the botanicals should therefore be applied early in the growth cycle of cowpea on or before the appearance of anthracnose symptoms on cowpea to minimize its spread and damage to the crop. The figures (Figures 1 and 2) further suggest that *A. cordifolia* and *T. pachysiphon* may be applied at higher doses to effect good control, unlike *L. camara* which could be applied at lower doses for effective control. Therefore, the closest alternative and/or substitute for the synthetic fungicide in this study is *L. camara*.

Frequent synthetic chemical sprays on cowpea and other crops to stem fungal diseases 2-3 WAI has also been reported (COPR 1981). Edema and Adipala (1994) and Oparaeke (2007) reported that weekly sprays with mancozeb or extracts of *P. guineense* on cowpea 2 WAP until flowering and podding significantly controlled brown rust (*Uromyces vignae*) and bruchids in the crop. In another study, Bretag (2008) found that for effective control of anthracnose disease of lentil, the fungicide Bravo 500 (50% Chlorothalonil) should be applied prior to onset of the disease and repeated a fortnight later. Awurum et al. (2016) reported that maintaining biweekly sprays of botanicals on onion and *Amaranthus* significantly checked the initiation, development, and spread of wet rot and anthracnose disease in the crops which corroborates the recommended control period at 4 WAP than 6 WAP for anthracnose of cowpea caused by *C. destructivum* in this study.

Time of application of chemicals and botanicals prior, simultaneous or after infection affects the efficacy of the product (Bretag 2008). Amadioha (2003) and Falade (2016) reported that the initiation, growth, and spread of anthracnose disease of cowpea caused by *C. limdemuthianum* in cowpea were checked significantly ($P \leq 0.05$) better with extracts of *P. nigrum, O. sanctum, D. strammonium, J. gossipifolia* and *T. procumbens* when applied 2 days prior to the inoculation or arrival of the pathogen on the crop than when applied simultaneously or 2 days after infection with the pathogen. Also, *X. arthiopica* and *A. indica* demonstrated higher fungitoxicity against the organism at 2 days before inoculation of the pathogen than at other application times (Amadioha and Obi 1998).

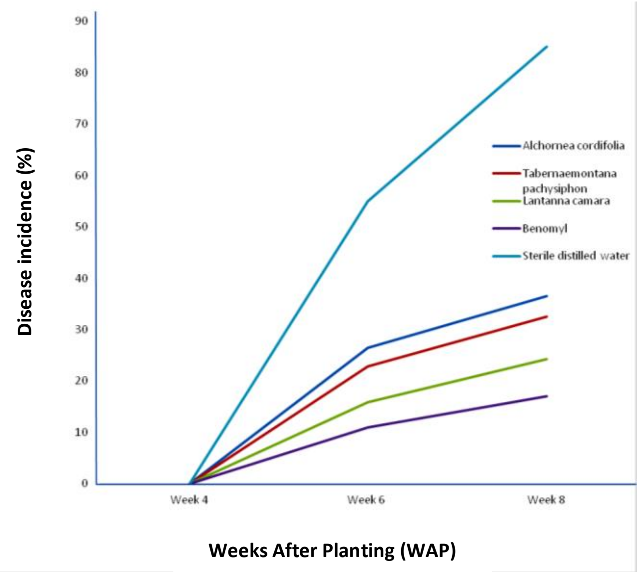

Figure 1. Mean effects of different concentrations of methanol extracts of the plant materials on disease incidence in cowpea 8 WAP

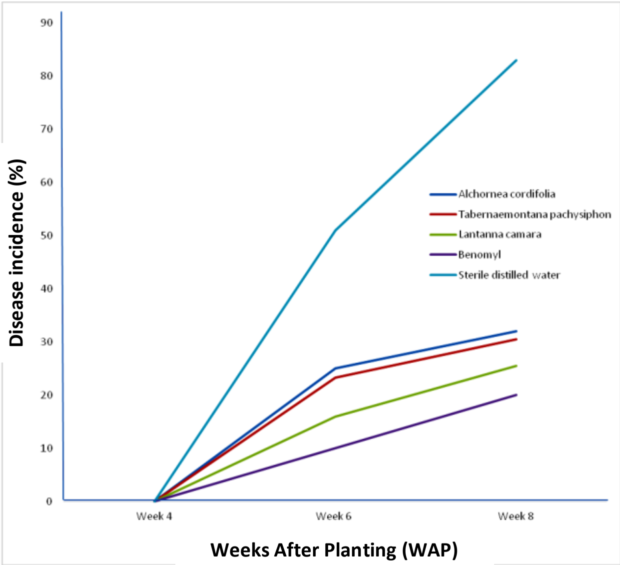

Figure 2. Mean effects of different concentrations of water extracts of the plant materials on disease incidence in cowpea 8 WAP

This suggests that plant extracts, besides direct toxicity to the test fungus, seem to stimulate host plant immunity towards resisting the disease and this may explain the higher control effects from the extracts of the test plants when applied at two or more days before than simultaneous or 2 or more days after inoculation of the pathogen on the crop. The less efficacy of the test extracts especially *A. cordifolia* and *T. pachysiphon* recorded in this study where the extracts and the pathogen were simultaneously applied on the test crop collaborate these findings.

The influences of phyto-pesticides in improving crop yields have been reported. Aqueous extracts of *Piper guineense* and *C. papaya* in a greenhouse trial on cowpea, besides reducing the development and spread of anthracnose disease caused by *C. destructivum* on the crop, improved the leaf area index, plant height, and biomass accumulation of test crop (Enyiukwu and Awurum 2013b). Also, extracts of *Hyptis marrubioides, Aloysia gratissima* and *Cordia verbenacea* significantly improved seedling emergence and dry matter content of treated soybean challenged by the anthracnose disease agent *C. truncatum* (Da Costa et al. 2012) Similar reports on field trial on *Amaranthus cruent*us (Amaranth) and *Allium cepa* L. (onion) exposed to phytochemicals derived from *Dennettia tripetala, Azadirachta indica and Spondias mombin* noted that besides significantly reducing the damage attendant from *C. curcubitarium* causing wet rot and *C. gloeospoirioides* incitant of anthracnose disease respectively on the crops, enhanced the yield and yield attributes of the test plants (Awurum and Nwaneri 2011; Awurum et al. 2016). According to Mark et al. (2015) essential oil derived from *A. sativum* L. effectively improved the number of leaves, leaf area, plant height and stem girth of treated cowpea. Findings from this study whereby the extracts of the test plants (*L. camara, T. pachysiphon* and *A. cordifolia*) significantly (P≤0.05) improved the plant height, number of leaves and dry matter yield of cowpea agree with the foregoing submissions.

The better performance of the methanol extract in improving the biomass content of the test crop over the water extracts (Table 2) may be due to a better extraction and synergism of the active compounds in the methanol, as extracting solvent rather than water. Or it may be that the active principles dissolved more or were more soluble in methanol, as extracting solvent than water which translated to better control of the disease and yield of the crop. It could also be possible that water extracts of the test plant contained more inhibitors to the active principle than the methanol extracts (Amadioha 2001). Similarly, the better performance of benomyl over the plant-derived phytochemicals may have been a result of its longer persistence in the eco-system when compared with the phyto-pesticides which are easily degraded by heat and UV radiation (Enyiukwu et al. 2014).

The improvement in the yield and yield parameters of the crop recorded in this study is thought to be brought about by two mechanisms-reduction of the growth and spread of the fungal pathogen causing the anthracnose disease and stimulating or priming of the crop immunity to resist further attacks by the disease-causing organism (Enyiukwu et al. 2016). Biopesticides derived from *Gliocladium virens* showed strong antibiosis against *Alternaria helianthi* evidenced by bursting of the hyphae of the pathogen and inhibiting its cellulose, cutinase and chitinase activities (Anitha and Murugesan 2012). Phyto-chemicals gleaned from *Dennetia tripetala, Azadirachta indica* and *Spondias mombin* improved the yield and quality of the treated crops by stemming the fungal attacks on the vegetables. These plant materials contain phenols and phenolic acids. Phenols are aromatic alcohols, which are constituents of various ranges of pesticides (Okwu and Njoku 2009; Enyiukwu and Awurum 2013a). In bio-systems this class of phytochemicals has been reported to block cell division, slow cellular growth and elongation, hamper sporangia formation, spore development and impair a wide array of microbial enzymes (Enyiukwu and Awurum 2013a). *Reynoutria sachalinensis* a phyto-pesticide containing the anthraquinone compound and is reported to induce defense mechanism in the host through stimulation and boosting of chitinases production, phytoalexin synthesis, papilla formation, vacuolization of infectious haustoria, inhibiting reactive oxygen species (ROS) and enzyme phenolic pathways (Lehnohof 2007). Likewise, Reboledo et al. (2015) in agreement reported that extracts of *Phyacomitrella patens* activated defense responses against *C. gloeosporioides* in susceptible hosts. The phenolic-like compounds of the extract inhibited the advancement of the pathogen by inducing certain genes coding for the formation and incorporation of protein-like polymers that re-inforced the host's cell wall. Also, bioactive fatty acids and essential oils containing functional groups or substituents such as furans, aldehydes, oxides, ketones, phenols, lactones, coumarins, and terpene hydroxides could induce resistance in treated crops through induction of host enzyme peroxidases against invading pathogens (Lemos da Silva et al. 2015). In beans (*Phaseolus vulgaris* L.), increased enzyme peroxidase activities due to fatty acid-containing phytochemicals have been associated with resistance to anthracnose disease caused by *C. indemuthianium* in the crop. The mechanism of this action has been linked to ability of extracts of *Cymbopogon flexuosus* and *Vernonia polyanthes* to induce host enzyme peroxidases to produce free radicals (H_2O_2) toxic to the pathogen in an oxidative burst and participate in lignin synthesis that strengthened host cell wall (Lemos da Silva et al. 2015). Applying phytochemical controls early enough was thought to have supplied phenols or improved the production of infection-fighting phenolics or radicals such as hydrogen peroxide in the onion plants through re-enforcing the structural components of its walls thereby reducing the advancement and damage due to fungal pathogens resulting in improved yield and quality of produce (Awurum et al. 2015). Therefore, the ability of the phytochemicals used in this study to improve the yield and quality of the treated cowpea may be due to these protective mechanisms developed by the host due to the phytochemicals from the test plant materials.

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Grape supply chain: Vertical coordination in Ningxia, China

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Abstract. *Li S, Bitsch L, Hanf JH. 2021. Grape supply chain: Vertical coordination in Ningxia, China. Asian J Agric 5: 12-21.* Since 2018, China has been the second-largest grape-growing country in the world, with 875 thousand hectares in total. A former study indicated a transformation within the wine grape supply chain management in the Chinese wine market, from traditional coordination to significant vertical integration. The Ningxia Helan Mountain's Eastern Foothill wine region (China) is considered to be the best wine region in China and has potential to lead domestic wine production. This study summarizes the typology of grape supply models in the Ningxia wine industry. We also examine influencing factors for the arrangements of current vertical coordination models of grape materials supply, particularly the hybrid organizations and contractual relationships are analyzed.

Keywords: China, political influence, supply chain management, vertical coordination, wine

INTRODUCTION

A stable supply of raw materials and quality of final products is crucial to various agricultural industries, particularly for the grape and wine industry. Theoretically, the problems of quality and/or quantity are supposed to be overcome through the application of vertical coordination. Wine grapes are perishable products, requiring close coordination between growers and processors during production processes. Therefore, numerous studies spend great effort on examining related motivations and various organizational arrangements of vertical coordination, exploring the roles of contractual relationships, especially in well-established European and American wine regions (Chambolle et al. 2006; Fernández-Olmos et al. 2009; Franken 2014; Goodhue et al. 2003). In addition, some research has considered the design and implementation of vertical coordination in developing wine regions (Chaddad et al. 2017).

Since 2018, China has been the second-largest grape-growing country in the world, with 875 thousand hectares in total. 163,000 hectares are used for table wine production (OIV 2019). The Chinese wine grape industry has a wide variety of vertical coordination mechanisms, a significant degree of product differentiation. The industry has variation in the product, size of business, and dispersed wine regions (Zhang 2018).

A former study indicates a transformation within wine grape supply chain management in the Chinese wine market, from traditional coordination to significant vertical integration. Currently, in production volume, productive partnerships are still dominant applied by medium to large-scale companies. The number of processors having vertically integrated vineyards now takes up a greater share, especially with boutique and emerging wineries in Ningxia wine region. Commonly, in the Chinese wine regions, a single wine producer adopts several coordination forms simultaneously. Existing studies revealed quality as the driving motivation for the adoption of tighter coordination. The used forms and mechanisms of coordination that are more specialized and tighter contractual relationships are interesting areas to be identified explicitly for the Chinese wine regions.

At the China Wine Summit Forum 2019, the Top 10 Chinese Wines of the Year were jointly recommended and awarded by wine critics Jancis Robinson MW, Bernard Burtschy, and Ian D'Agata. Among them, six wines were from Ningxia Helan Mountain's Eastern Foothill wine region (Ningxia region). The Ningxia region is considered to be the best wine region in China and has the most potential to lead domestic wine production. Therefore, it is reasonable to choose the Ningxia region to represent the quality wine regions. Due to differences in political backgrounds and market development processes, it is necessary to examine if the Ningxia grape market situation is in line with previous findings.

Through the conduction of qualitative research in the form of face-to-face in-depth interviews in Ningxia region and qualitative data analysis, this paper proposes to examine the structure and mechanisms of grape supply models. Specifically, to identify arrangements of contractual relationships in aspects of contract design, content, duration, contract enforcement, etc.

Theoretical background

In general, vertical coordination refers to the synchronization of successive stages in the vertical marketing channel from producers to consumers to overcome problems of supply and quality (Götz et al. 2009). It does not include transactions on spot markets, where the commodity exchange is based on a price agreement only. It includes both productive partnerships and vertical integration (Swinnen et al. 2007). The key for all types of vertical coordination is contracting. Marketing contracts only address the issue of supply disruptions by private contractual initiatives (Dries et al. 2005; Gow et al. 1998), whereas production contracts address quality concerns (Gorton et al. 2003). Meanwhile, contract enforcement is still one of the most severe barriers to successful vertical coordination (World Bank study 2005). In some cases, public enforcement institutions are not fully functioning and lack appropriate governance mechanisms. Two reasons for the breaching of contracts have been detected. First, producers mistrust their buyers and feel uncertain about committed payments. Second, they may not be able to fulfill a contract because contractors cannot offer basic inputs (Gow et al. 1998).

Consistent with the broad quality-coordination relationship, it is found that wineries employ different contract specifications to motivate desired behaviors by growers in different regions, which is also supported by Fraser (2005). According to existing studies, it is assumed that the higher the priority to secure quality and/or quantity of raw materials is, the stronger is the shift from spot market transactions towards advanced vertical coordination mechanisms. Goodhue et al. (2003) and Ewert et al. (2015) find that coordination that is more formal is associated with higher product quality. As noted by Fernández-Olmos et al. (2009), wineries that produce high-quality wines are more likely to integrate vertically than those producing low-quality wines. In line with these findings, a former study indicates that the type of vertical coordination in the Chinese wine grape supply chain has also experienced a transformation from random grape transactions to more complex and closer coordination. For example, there are more specialized contracted relationships between grape cultivators and wine processors. 'Dragon-head enterprises' or independent wine processors, who strive into the direction of vertical integration through different mechanisms led to this development.

Starting in 1978, the Chinese national government introduced a new era of modern agriculture, inducing a shift from small-scale to large-scale agriculture (Herma et al. 2017). 'Dragon-head enterprises' (DHEs) refer to large firms selected by the government since the 1990s to lead the drive for 'Agro-industrialization' and agricultural modernity. In this process, the government adopted the replacement of local markets with contractual farming, replaced dispersed small-scaled production (which is proposed as backward and unproductive) with large-scale agribusiness (which is proposed as efficient and modern) (Schneider 2017). In China, fruit production is dominated by small farmers because of the nature of land tenure (Huang et al. 2008). In detail, every farm family is given on average around 0.3-hectare land for agricultural cultivation, while the agricultural land in China is not considered as private property since rural collectives officially own land and manage land leases. As shown by Zhang (2015) and Ye (2015), there is a highly developed market for land use. By separating the land into ownership, contract, and use-rights, village collectives and individual villagers could transfer the right of use on a piece of land to another economic entity, while maintaining ownership and contract on the land.

The government supports DHEs to lead coordination relationships with farmers. With close ties to the regional government, they are also recipients of grants and policy support, and many are or have been at least partly state-owned. Some prior studies have illustrated that government interferences affect the arrangements of supply chains in China dramatically. The vertical integration led by DHEs was promoted aggressively by the government as the more favored form of vertical coordination, to realize industrialization in some agricultural production fields. For instance, grape transactions through contracted coordination with DHEs are more common in traditional Chinese wine regions like Yantai of Shandong Province. Vineyards here are farmed quite intensively and have been well established for a long time. Meanwhile, the Shandong Provincial government is one of the nation's leaders in following the central government's provision to select and support large-scale capitalistic DHEs, spending 50 million RMB each year in direct subsidies to select DHEs since 2002 (Report: 219). Aside from grape cultivation, some specialized farm cooperatives are organized by local county collectives in Penglai to better control the quality of inputs, offer advanced techniques for planting golden pears, and share market information with cultivators (Yu et al. 2009). It is shown by Huang (2011) that individual farm households benefit more in specialized cooperative coordination with private wine processors than compared to working with DHEs. Yet DHEs are overall bigger, more profitable, and more subsidized by the state. They are expected to cover larger numbers of farmers and create a greater economic performance. On the other hand, according to Huang et al. (2010) and Zhang (2012), in China, the cooperative organizations that are spontaneously formed by farmers have a hard time surviving. Reasons for that are the double risk of the market and the agricultural management due to their lack of leadership, internal cohesion, and strong external support (e.g., policy, finance, technique, etc.). In this case, it is more efficient than those formed cooperative organizations led by DHEs to realize large-scale agricultural production. The success of each of the models depends on the future choices of the Chinese government, which model will be promoted (Huang 2011). After reviewing existing studies about supply chain management in other Chinese wine regions, the role of the government is not marked as significant (Jin 2015; Dong 2017). Nevertheless, as other studies have shown, a strong influence of the national or regional government on the emergence of vertical coordination is possible (Ma et al. 2014). For this study, the government interferences on vertical coordination in the regional wine grape supply chains are examined within the Ningxia wine region. In the Chinese wine regions, grape material supply chain arrangements vary by wine processors. To a certain extent they depend on the production scale of the processor, the requirements on quality/quantity of the grape material needed, the degree of the local grape and wine market development as well as on the interferences of the local government on policies of grape supply chain management and strategies of local wine region development. For the Ningxia wine region, the evolved processes of the models and organizational mechanisms of vertical coordination have not been identified yet. In terms of contract enforcement during the grape supply process, previous studies in the Wuwei wine region of Gansu Province (Jin 2015) and in the Xinjiang wine region (Dong 2017) indicate that some cases of farmers breaching their contract were detected, which led to the failure of the contractual relationship. As a result, local wine industrialization level cannot be improved. Partners must overcome problems of uncertainty, opportunistic behaviors, and contract enforcement among vertical coordination to produce higher quality products. Thus, vertical integration is most often introduced if these problems cannot be overcome. Therefore, arrangements of the contractual relationship, contract design, and contract enforcement problem in Ningxia are also crucial points for this study.

Grape and wine market in the Ningxia Helan Mountain's Eastern Foothill wine region

The Chinese wine production is transferring from a quantity orientation towards a more quality-oriented production, especially in some of the emerging quality regions. Ningxia region is often considered the best wine region in China nowadays and can lead the domestic wine production quality-wise.

The Ningxia Helan Mountain's Eastern Foothill wine region has just started to develop for the last few decades. In 1984, the Ningxia Agricultural Reclamation Yuquan Winery was the first winery to be established in Ningxia. Since then, Ningxia began to plant grapes on large scale. The Yuquan winery (now named as Xixia King Winery) belongs to the Ningxia State Farm, which is the biggest wholly state-owned agricultural enterprise in Ningxia. During the early development period around the 1990s, very few wineries were established to ferment the grapes. The bulk wines produced in Ningxia were transported to Shandong and Hebei provinces for blending. The wines were sold under brands of large enterprises, which were mainly COFCO Great Wall, Changyu, and Dynasty. The urbanization process in China and the expanded cultivation area of economic crops increased the cost of farming land; also, the labor cost, especially after 2008. All these economic and social factors motivated the movement of the Chinese wine-producing industry from the Eastern coastal regions to Northwestern China.

The wine grape planting area in Ningxia increased from 2.66 thousand hectares in 2003 and has reached 38 thousand hectares in 2018 (figure 1), becoming the largest concentrated wine production area in China (Zhang 2018). Up to now, there are 198 wineries in this region. Among all these wineries, 86 wineries were finished and their products could be seen in the market, while another 112 wineries were built but they were still under construction. Domestic and foreign big wine companies such as COFCO Great Wall, Changyu, Pernod Ricard, and Moet Hennessy Domaine Chandon have established wineries in Ningxia. Their scale and the characteristics of the company affect the wine producer's requirements of grape quality and quantity. Thus, in this study, the wine producers are separated into two major groups to be discussed: 1) 'Dragon-head Enterprises' (DHE) and 2) other wine producers. Firms like Changyu Pioneer Wine Company, COFCO Great Wall Wine Company and Xixia King Winery are examples of 'Dragon-head Enterprises' in the Ningxia wine sector.

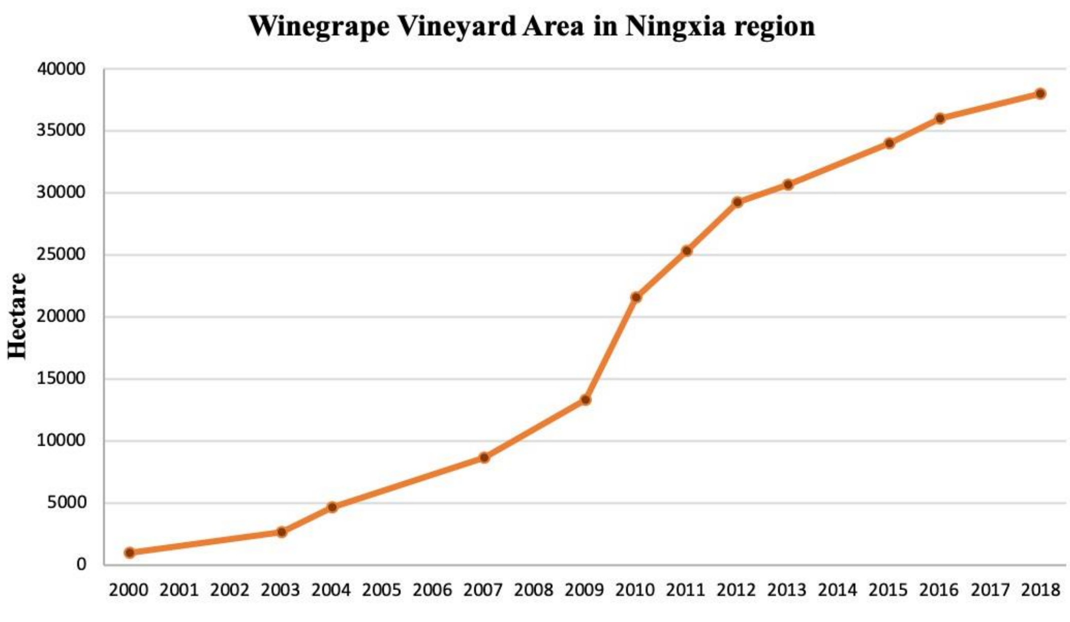

Figure 1. Development of vineyard Area in Ningxia region. Source: Zhang (2018)

Table 1. Overview of the major selected interviewees in Ningxia

Interview groups	Subgroups	No. of active companies	No of inter-viewers	In percentage
Government institutes	Ningxia Wine Bureau	1	2	/
Education institutes	Universities	2	2	100%
	Research institutes	3	1	33%
Wine producers	DHEs	3	3	100%
	Other wine producers	83	8	10%
Grape suppliers	Farmers	/	4	/

Under the guidance and support of the government departments, the wine industry in Ningxia has made great progress since 2010. To increase the value of grape growing and to face the challenge from the Chinese giants and imports, local authorities in Ningxia decided to adopt the "estate wine, regional brand" strategy for the local wine industry. That is to encourage wineries to possess private vineyards and make quality wines with their harvests to introduce more wine brands into the industry, making full use of the strong ambition of each producer to succeed in specific market segments. The massive production of standardized products was not welcomed at that time, as it is often regarded as cheap and inferior in quality. With the growing income and accumulated wine-consuming experiences, Chinese consumers are looking for domestic wines with good quality, attractive stories, and more accessible interactions. Small-scaled boutique wineries could better meet these requirements. In a short term of five years, Helan Qingxue, Silver Heights, Kannan Winery, Moet Hennessy Domaine Chandon, and several other brands have gradually built their reputations both domestically and internationally, by being well-known for their delicate estate wine production. However, since last year new investments in boutique wineries are not favored anymore by the government, due to some modifications on the policies concerning vineyard investment and winery establishment. That is also the reason why some wineries are still not finished. They cannot get construction permissions from the government to proceed. Meanwhile, the government collects grapes from their dispersed vineyards to be fermented by other large-scale wine producers. These adjustments in policies tend to strengthen the vertical integration in the grape supply chain even more.

Aside from grapevine growers, there are groups that need to be considered: wine producers, government institutes, educational institutes, and other active actors in the Ningxia grape market. Professional instructions, as well as their supportive roles on grape cultivation and vineyard management, may affect the arrangements and/or mechanisms of the grape supply models in the local region.

During the development of the Ningxia grape and wine market, grape supply models' arrangements have also gradually evolved to balance the requirements on grape quality and quantity, improve the production efficiency, and better adapt to market and the developments of the companies. Nevertheless, there are only a few relevant studies about the organization of the grape market and vertical coordination applications. Years ago, some studies about the Eastern wine regions had been conducted, but the results cannot be applied to the Ningxia region due to different market environments. Thus, it is worth examining the Ningxia grape market to identify the vertical coordination mechanisms while considering the specialties of this region. Our findings help to understand the structure of the Ningxia grape market and to derive implications for the optimization and development of the local wine region.

MATERIALS AND METHODS

Methodology

The study used an exploratory qualitative research approach. The qualitative research approach helps to extract patterns of meaning based on the collected data. Qualitative research is about recording and analyzing to uncover the deeper meaning and significance of social behavior and phenomenon (Creswell 2003). The data collection and analysis approach is methodical, but allows for greater flexibility than in quantitative research. The data collection of this research consisted of field research, particularly in-depth interviews were carried out.

For this study, the approach of a face-to-face, semi-structured individual in-depth interview was adopted to collect the required data. Face-to-face semi-structured interviews perfectly fit this study, offering the possibility to collect longer descriptive answers in a relaxed and trustful atmosphere. In total 20 interviews were conducted in Ningxia region, starting from 27th of March 2019 to 15th of April 2019. The interviews were conducted face-to-face in Ningxia, mostly in the working office of the respondents. The native language of the researcher and interview partners was the same, thus making it ideal and easy that all interviews were carried out in Chinese. Concerning the topic of this study, a set of predetermined questions were fixed as the interview guideline and respondents were encouraged to answer in their own words based on their experience. Almost every interview was recorded with the oral approval of the interviewee. On average, each interview lasted about 45 minutes. Table 1 shows a general picture of the 20 selected interviewees. All interviews have been transcribed. Qualitative data analysis after Mayring (2015) was applied.

RESULTS AND DISCUSSION

According to our findings, government interferences play a determining role in Ningxia grape and wine market development. It is logical to first understand the influences of the Ningxia authorities on the evolution of the grape supply chain. Secondly, the existing vertical coordination models in the Ningxia grape market are illustrated. Accordingly, the factors driving wine producers' to different choices and arrangements on grape supply are clarified. In the last section, the contract design, and problems of contract enforcement in contractual production relationships are evaluated.

The influences of the government on vertical coordination in the Ningxia wine industry

In the Ningxia region, the Bureau of Ningxia Grape Industry Development (Wine Bureau), as a governmental institute, is responsible for the grape and wine market. It is the office of the Administrative Committee of Grape Industry Zone of Helan Mountain's Eastern Foothill Wine Region in Ningxia; it is the first provincial government wine department in China. It works as a branch of local authorities which (i) carry out industrial development plans, (ii) formulate strategies and standards, (iii) solve land management, and (iv) coordinate among growers, producers, and distributors. The Wine Bureau has great impacts on the evolution of local grape supply models and vertical coordination mechanisms.

The government and local collectives take the role of land agencies. Since no individual in China owns their land (land belongs to the village and is contracted to households for 30 years for no rent), all farm households have access to their land (Rozelle et al. 2006). According to the interviews with the government institutes, a large area of vineyards was integrated by local collectives after the year 2000 and then reallocated to DHEs and wine producers who applied for land of vineyards. When the 'estate wines, regional brand' strategy was chosen by Ningxia authorities around 2010, several of the boutique wineries were attracted to this region. According to our interview sample, five of 11 wine companies were established around 2010. Three of these five companies mentioned that they applied for the land of vineyards from the government. The using right of the land was then transferred to the company by paying the rent to the government and signing a land lease with a duration of 30 years. For the other two companies, one rented the land from the Ningxia State Farm, and another one rented the land from the rural collective.

Following an official document issued in 2016 by the Ningxia authorities, one of the requirements for the application of establishing a boutique winery was to possess private vineyards of at least 15 hectares. Besides, supportive policies for encouraging vertical integration of grape supply chain are also presented in the document: *'To encourage vertical integration between vineyard and cellar management: the government would integrate part of the existing vineyards from companies/farmers who purely work on grapevine cultivation, meanwhile domestic and foreign companies (investors) are encouraged to establish wineries with existing vineyards. New investors and original vineyard owners (companies or farmers) form a consortium of interests by means of land auction, shareholding, renting, transferring, or contracting, etc. New vineyards can be applied for allowances for the first three years. There are subsidies for wineries that vertically integrate vineyard and cellar management. From now on, enterprises and farmers are not supported or encouraged to purely grow wine grapes.'* (Ningxia Wine Bureau & Ningxia Provincial Department of Finance 2016). These policies promoted the rapid transformation to vertical integration in the grape supply to better control the grape quality and to make unique quality wines and implement the 'estate wines, regional brand' strategy.

However, now the policy direction changed towards the 'Great Item' strategy starting with the renewed document in 2018. Since 2018, it has been hard for small- to medium-sized boutique wineries to apply for land from the government. Besides, there are some boutique wineries that just established vineyards two or three years ago, while their cellar's construction permission is still not granted by the government. Due to the changes in policy, only companies who can comply with the capacity to produce wine from at least 135 hectares would be permitted to be established (Ningxia Wine Bureau & Ningxia Provincial Department of Finance 2018). This change is also confirmed by the establishment of the 'Xige Estate' who owns 1,267 hectares of vineyards and the appearance of a wine brand 'Helan Hong'. This brand integrates many grape bases, which all have strict managing standards. 'Helan Hong' grapes are now fermented with grapes from more than 2,000 hectares of vineyards. In the aspects of marketing and brand promotion, all the financial supportive policies for individual wineries or enterprises are canceled. The Wine Bureau is organizing promotions on the great regional brand 'Helan Hong', which is expected to represent Ningxia wine. These modifications in policy push the industry to further vertically integrate with regard to vineyards. Thus, the policies and strategies issued by the government have determined the grape supply models and the direction of the industry's development to a great extent. It can be said that the current situation of the vertical coordination within the Ningxia grape market was determined by the political influences of the government.

Existing vertical coordination models in the Ningxia wine sector

Summarizing from all the 20 interviews, several organizational models of the grape supply of wine processors are discovered. Based on the data analysis results, there are mainly five existing grape supply models. Below is a description of the vertical coordination mechanisms of these five models in the Ningxia region:

First. Dragon-head enterprises (DHEs) vertically integrate farmers' vineyards to establish standardized vineyard bases. The standardized vineyard bases are completely controlled by the DHEs specialist's management, with relatively strict quality control mechanisms. These grapes from their own vineyards are used to make estate wine. Generally, the land for standardized vineyards is collected by the local rural collectives or the government and then reallocated to DHEs and/or large-scale firms. The using right of land was transferred to the DHEs, while the farmers, as the original owner, are paid with the rent; it is also common for the farmers to continue working in the vineyard or the cellar as an employee. During the development of the Ningxia wine industry, vertical integration was used to emphasize the concept of 'estate wine', maintain the order of the market, and decrease the loss of farmers. In other words, vertical integration emerged due to a greater demand for higher quality products in a more developed market, which corresponds with the expectation that the degree of market development is important for the degree of vertical coordination.

Second. Dragon-head enterprises also work on contracted vineyards with grape suppliers by using five to ten years long-term contracts, which regulate the requirements of quality and quantity, as well as the agricultural assistances offered by the enterprises. Specialists from the company and/or education institutes are sent to the farmers to give cultivation instructions during the growing season. The price settled by the DHEs is considered a benchmark for other transactions of grape procurement. The government subsidizes electricity and/or irrigation systems, grapevines, and vineyard construction in the first three to five years; in some cases, farmers and/or firms receive cash directly. For DHEs, the grapes from contracted vineyards are mainly used to make table wines. Contract enforcement is not a problem for this model, except for some farmers who complain about a low income. In this case, the contractual relationship between DHEs and grape suppliers is relatively stable. Farmers believe in the enterprise and rely on the enterprises' stable demand for grape materials to maintain a risk-free income. At the same time, DHEs need grapes from contracted vineyards to keep consistency in their production. Thus, these contracts tend in the direction of vertical integration because the firms almost completely control the vineyards.

Third. Wine producers integrate the farmers' vineyards into their vineyards by renting land from farmers or collectives. The area of private vineyards varies between companies with different economic scales. Generally, the outcomes from the private vineyards account for 80 to 100% of the company’s whole production. The vineyard management is fully controlled by the experts or the winery managers. Seasonal vineyard workers are hired from surrounding villages. The local government supported the villagers in moving from poor mountainous areas and settling in emerging wine towns close to the wine companies. The quality of the grapes from individual vineyards is at a high level and these grapes are used to produce high-quality estate wines. In Ningxia, the private vineyards of some emerging wineries are allocated to the government allowance projects.

Fourth. For some wineries, 10 to 20% of their whole production relies on contracted vineyards through contracting with grape suppliers in the form of mid-term contracts, annual contracts, or oral agreements. These different organizational forms of contracted vineyards belong all to productive partnerships. Mid-term contracts are always used by boutique wineries, which are booming in recent years. They have strict requirements for high-quality wines. These wineries require mature grapevines or grapes with specific characteristics that are missing in their grapes. The grape supplier manages the vineyards following the clauses in the contract. Some companies would participate in decision-making and guide growers during the growing season. On the contrary, short-term contracts or oral agreements could be marketing contracts. They are more applied by wine producers who consider securing the quantity of their production with the grapes from contracted vineyards. The agreements address mainly the harvest schedule, quantity of grapes, and payment method. The required quantity may vary in each vintage due to concerns of storage, market expectation or extra orders from customers, etc. For them, the quality of these grapes is not a big issue, and they have no pressure on finding a partner who owns the required number of grapes. Thus, the flexible or rather loose contractual relationship fits better with their requirements. The expectation in theory framework is verified: the design of partnership differentiates in contract duration and the themes in production contracts and marketing contracts, depending on the strategy of the contracting initiators.

Fifth. The last option we found is grape procurement on the spot market during the harvest season. The grape’s quality cannot be guaranteed. These grapes sometimes are used to make up for the shortage of production for some wineries in particular vintages, which may be caused by extreme weather, damages, or unexpected additional orders, etc. Besides, DHEs assume the social responsibility to deal with the excess grape materials at the end of the harvest season.

Summarized from all interviewed wine companies, the wine producers in Ningxia usually do not only apply a single grape supply model. Almost all the wine companies are applying multi-supply models. The ratio of each model varies between the companies, depending on the producer's decision, product strategy, and the demand of the market. Wine companies in Ningxia prove this. Among all interviewed Ningxia wine producers, only two rely entirely on private vineyards and one innovative wine producer simply obtains grape materials from contracted vineyards. Briefly, every interviewed wine producer in the Ningxia wine region, except the innovative wine producer, possesses private vineyards under strict control and is used to make quality estate wines. Aside from the crops in their vineyards, grapes are at the same time supplied by contracted vineyards or procured on the spot market. For some DHEs, private vineyards, contracted vineyards and spot market procurement are coexisting. The current situation was gradually formed under the influences of politics and market response during the evolution of the Ningxia wine industry’s development.

Influencing factors on the formation and selection of vertical coordination models in the Ningxia wine grape market

Due to the differences in the company’s characteristics, the scale of the company, local policies, the requirements for grape quality/quantity, and strategies of marketing, the wine producers apply distinct supply models to manage their grape materials.

The characteristics of the companies are essential factors for the decision of raw material structure and sourcing models. The dragon-head enterprises are those companies defined by the country and they are assigned with responsibility for driving the local industrialized economic development. All interviewed DHEs mentioned that the company has social responsibilities: (i) including assisting farmers with the reclamation of wasteland into vineyards, (ii) providing references for the formulation of the Chinese wine quality standards, (iii) solving excess resources through direct procurement at the end of the harvest season, (iv) improving the infrastructure in surrounding villages to facilitate the lives of villagers, etc. In order to take full advantage of the superiority of the wine region and to ensure a constant supply of high-quality grapes, the companies have been committed to constructing standardized vineyard bases with the farmers, which is the primary motivation to apply large-scale vertical integration.

As a result of the large demand for their products, two of three DHEs in Ningxia have vineyards above 1,000 hectares and possess large production capacities. This extraordinary large-scaled production has pushed DHEs to be the first and the largest group which needs to be considered while talking about grape supply arrangements. They perform direct grape procurement, contractual partnership, and vertical integration of farmer's vineyards. Four of eight wineries own vineyards of around 20-35 hectares for other wine producers. Except for grapes from their vineyards, three of these four wineries buy in about 10 to 20% of grapes from external vineyards. The amount and required quality of the grapes vary by the demand of the winery for each vintage. There are two wineries owning vineyards of 60 to 150 hectares and two possess vineyard areas of more than 1,000 hectares. None of them needs to buy grapes.

In the Ningxia wine sector, local policies determine the organization of the grape supply models to a great extent. A wide distribution of viticulture appeared, and extensive vineyards were established under governmental support, which aimed to develop agriculture on wastelands and help residents overcome poverty. At that time, the Ningxia region was just a region producing grape materials for mass production in other wine regions. Since the 2000s, the integration of farmers' vineyards has been organized by DHEs together with the government to drive the 'Agro-industrialization' and the agricultural modernity in the wine sector. Around the year 2010, specialists and investors revealed the great potential of Helan Mountain Eastern Foothills wine region. In order to follow and respond to the government's 'estate wine' strategy, all 11 interviewed Ningxia wine producers, except one, possess integrated vineyards and grapes from individual vineyards. These vineyards are all used for their estate wine production.

Supportive policies were issued to promote integration in grape cultivation and attract new entrants. The interviewees demonstrated that their vineyards are either integrated backward by paying rent for farmer's land (around 1,200 Euro/ha per year) or they applied for the land from the government through signing land leases. Regarding the management mechanisms of vineyards, the company can fully manage the private integrated vineyards. Vertical integration is the most effective form to overcome the quality/quantity problems within a single firm. The research expectation is confirmed. The determining role of politics has shown stronger power in recent two years. Two of the wine producers interviewed established the production facilities in the last two years. Both own more than 135 hectares of private vineyards (partly applied from the government) and they are preparing for their expected huge production capacity. Due to the modification in policies, further vertical integration is expected.

As specific requirements for target products, grape quality and/or quantity, are considered. These goals are achieved by either specialized management within the individual vineyards or via collaboration with contracted vineyards. As stated by the companies who employ experts to manage their private vineyards, the aim is to produce higher quality grapes. Meanwhile, more than half (eight) of the interviewed companies rely on contracted vineyards. For the two DHEs, they have more contracted vineyards than their own vineyards, aiming to provide bulk wine for other product lines, keep the consistency of their final products, and make up for insufficient yield same time assume their social responsibility in poverty areas. While for the other five wine producers, the reason to rely on contracted vineyards is to meet unique grape quality requirements (e.g., variety, acid levels, tannin structure, etc.; around 10 to 20% of their production relies on contracted vineyards. In the first ten years after the establishment of the vineyard, the grapevines in their own vineyards are too young to make the targeted wine, which is expected to have great maturity and complexity. Besides, some of the interviewees stated that the quality of their own cultivated grapes is not necessarily better due to the shortcomings of terroir conditions. Likewise, some wine producers search for grapes with more fruit flavors and better health conditions. In addition, the popularity of some favored grape varieties, like Marselan, is another motivation for wine producers to contract with a new partner for the long-term.

Contract design and contractual obligations in the Ningxia wine sector

As mentioned above, around 70% of the wine producers rely on contracts and agreements. Details about the contract design and contractual obligations were examined with the interviewed wine producers and grape suppliers.

There are several forms of contracts and agreements between grape suppliers and buyers. A type of marketing contract is signed during the summer until the beginning of September. In this case, buyers go to farmer's vineyards to select grapes and/or plots. They decide on the required quantity and the final quality (often judged by the sugar content) which is fixed in the contract. Concerning the 'price criteria', two DHEs set the lowest price as a benchmark and other buyers take the same prices or prices above the benchmark. In general, the prices vary between grapes in different vineyard locations and varieties. For the wine grape, the price relies on quantity, sugar level, ripeness, polyphenol compounds, healthiness, and other quality indicators. It ranges between four to ten RMB per kilogram. The price criteria also differ greatly between wine producers. Some wine producers have strict quality requirements and are willing to pay a higher price only if the grapes meet their requirements. The requirements regarding the quality can sometimes not easily be measured and this is in line with the findings from prior studies.

The cooperation model is different for the farmers who own vineyards that are positively recognized by producers, as they have the potential to produce top-quality grapes or cultivate popular grape varieties. In this case, wine producers are more in favor to use mid-term contracts. Six producers stated that they work with two to five-year contracts. Normally, producers would renegotiate the agreements with farmers each year and make clear which is the required quantity and quality for this vintage. The clauses in the contracts differ between wine processors. Processors pre-pay fees to grape suppliers in spring. Some processors ask to be informed when the grower plans to use pesticides and the experts from wineries always join the canopy management during the growing periods. Some provide grapevine material to growers and offer training services of advanced technical operations for grape cultivation as farm assistance. However, no interviewed wine producer would provide fertilizer, pesticides, machines, or other agricultural materials to farmers. If extreme weather or disasters happened during the growing and/or harvest season, the growers mostly bear the losses, but the buyer provides some necessary support to reduce the loss. For the price statement within the short-term contracts, wine producers would pay for the final quality of the grapes. For grapes far from the required quality, producers pay the supplier with the market price. If the grapes meet the required quality, the payment is the price that was agreed to in the contracts or an even higher price. In this contractual relationship, the contractor provides a market for the goods, engages in many of the grower's decisions, and retains ownership of important production inputs, which is in line with our research expectation.

While a five to ten-year long-term contract is only found with DHEs, it is the most common form for DHEs to work with contracted vineyards. Like the clauses of other contracts mentioned above, the yield and required quality are regulated. The enterprise always sends experts to the farmers during the growing season to give cultivation instructions and give guidance for the correct treatment of the vine. The income of contracted farmers mostly depends on the sugar content and the weight of the grapes. Due to huge demand, a great reputation, and the large economic scale of DHEs, farmers are subsidized in the first several years to collaborate with DHEs and do not need to worry about contract enforcement. Nevertheless, many farmers complain about the price, which is always lower than the market price. This production partnership is close to vertical integration under outer political pressure.

In terms of contract enforcement, as mentioned by some producers, they must offer some extra benefits to farmers to keep the contract running. Most of the wine producers, except one, state that a breach of contract has never happened but they heard that cheating of farmers was common some years ago. The experts from educational institutes feel that contract enforcement is still a big challenge. There is no specific legal protection or enforcement or supervising department. Some deviant behavior of farmers does not match with clauses in the contract and sometimes, a breach of contract occurs. One producer shared their experience: 'In great vintages, farmers disregard the contract and sell to buyers who promise higher prices; while in poor vintages, they may come back to the suppliers, forcing them to take grapes and pay the price; agreed on in the contract.' Talking to farmers about contract enforcement revealed that some independent farmers refuse to work with contractual relationships: they do not believe that wine producers are always trustworthy. Some deviant behavior of wine producers is given. For example, it is hard for farmers to ask for compensation from companies for poor vintages; wine companies do not pay the price as they promised in the contract: the companies reduce the price regardless of the income of the farmers or the wine companies delay the payments, etc.

A conflict of interest can breed mistrust and stagnation, ultimately contributing to the failure in fulfilling the contract. No relevant agencies or departments supervise the effectiveness of the contract. Contract enforcement entirely relies on the consciousness of both partners and their emotional connections. The risk of contract enforcement is a reason for the preference for private vineyards.

Discussion

This study has examined the structure and organizational form of vertical coordination mechanisms in the grape supply chain, as well as possible determining factors for the evolution and arrangements of vertical coordination in the Ningxia wine sector. One of the main findings is that almost all of the wine companies are applying a multi-supply model. Spot market transactions are used to secure basic range products while for higher quality products, the vertical coordination mechanisms advance more towards vertical integration. This confirms the assumption, that the higher the needed quality, the tighter the chosen coordination mechanism.

The ratio of each model varies between companies as it depends on product strategies and market demand. It has been evident that politics and market responses hugely influence the current situation during the evolution of the Ningxia wine industry development.

In the Ningxia wine sector, politics influence the shift from uncertain random transactions on spot markets towards vertical coordination mechanisms in the grape supply system. After collecting dispersed vineyards in the early stage, DHEs are first asked to conduct vertical integration with farmers. Since 2010, when the 'estate wine' strategy was promoted, integrated private vineyards are then supported and motivated to be applied with emerging small- to medium-sized wine processors. However, policies and strategies concerning the regional wine industry development have shown some modifications since 2018. The 'Great Item' strategy has been adopted. New entrants with the scale of boutique wineries are no longer favored, but large-scale producers are welcomed, and the regional brand is strongly promoted to improve the power of the regional brand and to increase market share. A larger share of vineyard bases have been integrated for the regional brand and enterprises and are managed by international teams under high standards to keep quality but also improve the cost performance. Therefore, this adjustment has further accelerated the vertical integration of grape raw materials.

As a result, of the market response, business relationships between grape suppliers and wine producers were progressively induced from spot market procurement to vertical coordination. Since 2010, many wine producers entered Ningxia, which led to a rise in grape prices and the spot market for grape material was extremely frequented. However, the depressed market in 2013 combined with the lack of market experience of new companies. This led to difficulties in product sales, which consequently ended in a decline in the demand for grapes and therefore in a price decline until 2016. During that time, numerous arguments came up due to a breach of contract from either the grape growers or the companies. The market pressure forced many grape growers to exit grape cultivation, thus the government had to integrate their vineyards to save and keep the grapevines. Contract enforcement problems caused hesitations on both sides of the contractual relationship, making them afraid to touch productive partnerships again. Nevertheless, contracted vineyards are still common for DHEs and some wine producers. DHEs have long-lasting partnerships with their contracted farmers to guarantee consistency in the quantity of their products. However, contracted vineyards have increased in recent years, as the wine market and consumers have become more mature and there is more awareness to look for high-quality wines. As shown in our results, some wine producers start to discover more top vineyards, engaging in specialized investments into vineyards, realizing the importance of maintaining stable and close coordination with grape suppliers. Overall, the findings are in line with other studies (Goodhue et al. 2003; Fraser 2005; Fernández-Olmos et al. 2009; Franken 2014; Chaddad et al. 2017). Contractual coordination and vertical integration are driven by the demand for quality wine production. The arrangements of the contractual relationship are mainly decided by processors. The decisions are influenced by the production scope and the marketing strategies of the processors. The contracts show differences in contract duration, agricultural or financial assistance from buyers, involvement levels in viticultural practices, and grape quality measurement methods. As stated above and in the theoretical background section, the forms of vertical coordination in the Ningxia region are dramatically affected by local policy. At first, vertical integration was promoted to make foster quality production and now vertical integration is used to support capital enterprises (DHEs) to form large-scale businesses. The topic of contract enforcement confirmed the same findings as in other regions. The lesser an environment is institutionally developed; the legal protection of contractual rights cannot be guaranteed. However, there is progress going on in the Ningxia wine region.

Conclusion

In this study, the required information was collected through conducting face-to-face semi-structured individual in-depth interviews with 20 selected participants. This study summarizes the typology of grape supply models in Ningxia wine industry and examines influencing factors for the arrangements of current vertical coordination models of grape materials supply in Ningxia region, particularly the hybrid organizations and contractual relationships are analyzed.

In line with prior findings is that vertical integration of grape cultivation increases due to the recognition and desire for wine producers to produce quality wines. Meanwhile, the local political regulations and the guidance of the government are crucial for the preferences of individual vineyards. The possibility of governmental interventions in the grape supply chain has been realized since the land ownership in the Ningxia region. The role of political institutions in the development of the Ningxia wine industry cannot be ignored.

On the other hand, for productive partnerships, long-term contracts are more frequently used by dragon-head enterprises to keep consistency in production. While some boutique wineries prefer mid-term contracts with high requirements on grape quality. In short-term contractual relationships or oral agreements, the quantity of grapes is more important for buyers than specific requirements on grape characteristics. In this case, grape suppliers have no bargaining power over prices. The wine producers dictate prices. For a more detailed analysis of the contract design, a much richer data set from wineries needs to be collected and matched to the specific grower requirements.

As illustrated above, to produce high-quality products, vertical coordination partners must overcome the problems of uncertainty, opportunistic behavior, and contract enforcement. However, within the less developed institutional environment in Ningxia, the problem of contract enforcement cannot be completely overcome. According to the interviewed wine producers, contracts can proceed smoothly relying on a smart cooperative partnership and a well-balanced relationship maintained by the partners themselves. Rather than legal concerns, the emotional connection between partners is one of the key factors for guaranteeing contract enforcement.

Considering the possible trend in a contractual relationship, the problems of contract enforcement need to be overcome in the long run. The government must establish a well-organized institutional environment, taking charge of the role of supervision on negotiations about contract enforcement. It is also critical to improve the wine law and ensure its implementation. A grape cultivation association could be organized and authorized to participate in the grape value determination process during the grape procurement to guarantee proper profits for grape suppliers. The bargaining power of independent farmers is low. Independent farmers are too dispersed; they cannot request a higher price. As shown in the results, the farmers working on mid- to long-term contracts with wine producers is better compared to other farmers. In this case, the establishment of a contractual relationship is suggested to farmers to decrease the risk of the transaction and offer more protection for their business.

For future research, the relationships between grape suppliers and producers should be analyzed in detail. Besides, the means applied by wine producers for strengthening connections and maintaining smooth contract enforcement have not been investigated. These would be topics worth conducting in future research.

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Growth performance of novel food based on mixture of boiled-dried granulated *Tenebrio molitor* larvae and date-fruit waste in broiler chicken farming

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Abstract. *Debache K. 2021. Growth performance of novel food based on mixture of boiled-dried granulated* Tenebrio molitor *larvae and date-fruit waste in broiler chicken farming. Asian J Agric 5: 22-28.* The present study was conducted to evaluate the growth performance of a new diet based on mixture of boiled-dried granulated *Tenebrio molitor* larvae (Tm) and date-fruit waste (Dw) in broiler chicken diet. A total of 56 two-day old broilers were randomly allotted to 4 dietary groups each with 2 replicates consisting of 7 broilers (C1, C2, Diet1 and Diet2). Equal mixture of three commercial cereal-based diets (chick starter feed, chick grower feed, and chick finisher feed) was formulated. The first control (C1) was 100% the commercial mixture. Second control (C2) is obtained by mixing 50% commercial mixture with 50% Dw. While the other groups (Diet1 and Diet2) were formulated by adding three ingredients at different proportions: 50% commercial mixture: 40% Dw: 10% Tm (Diet1) and 50% commercial mixture: 10% Dw: 40% Tm (Diet2). After the evaluation of daily body weight, clinical signs, specific growth rate and other clinical tests, the chickens were slaughtered at 60 days. Hematological, biochemical, copro-parasitological, and bacterial investigations were performed based on samples taken day 60. Weight gain of broilers fed with Diet1 was almost like broilers fed with first control (C1) diet. However, those fed with Diet2 were significantly ($P<0.05$) higher than all other dietary groups (C1, C2 and Diet1). Hematological and serum biochemical traits showed no dietary adverse effect, and copro-parasitological diagnosis was negative in all different dietary groups. Moreover, similar microbial communities were detected in digestive system parts of the same animal, no matter in relation to Tm inclusion or no. In conclusion, the overall results collected in this current study propose that date-fruit waste could be used as an exclusive feed for *T. molitor* insect rearing and dietary inclusion of mixture Dw-Tm into broilers meal could become a partial substitute for commercialized cereal-based diet without affecting the health of broilers.

Keywords: Broiler, date-fruit waste, insect meal, growth performance, *Tenebrio molitor*

INTRODUCTION

Chicken meat and eggs provide high-quality protein and are a source of all essential amino acids required in the human diet. They contain both saturated/unsaturated fatty acids, minerals, and sufficiently high quantities of all essential vitamins except vitamin C. Chicken is not only healthy meat, but also is relatively inexpensive versus other livestock meats, even though it contains more protein and less fat than red meat (Kralik and Kralik, 2017). Cereal-based meals are the most used vegetable protein source in diet formulations for broiler chickens due to the high level of protein (Veldkamp et al. 2012). However, the increasing cost and the negative environmental impact of cereal cultivation have led to the need for other useful animal nutrition alternatives. Insects (larva, pupa, and adult stage) including *Tenebrio molitor* larvae, commonly known as yellow mealworm, have been suggested in poultry feeding of their natural consumption by wild birds and free-range poultry (Zuidhof et al. 2003; FAO, 2013; Debache, 2017). This is due to their protein richness, lipids and essential elements, which were evaluated (dry matter) as follows: 22 to 48% protein, 15 to 38% fat, 4% carbohydrate, 58% moisture, 11 fatty acids, 20 essential amino acids, niacin, pyridoxine, riboflavin, folate and vitamin A, C, E, B1, B6 and B12 (Jones et al. 1972; Nowak et al. 2016; Payne et al. 2016; Rumpold and Schlüter, 2013). Moreover, Dobermann et al. (2017) reported the following mineral content expressed as mg $100g^{-1}$ dry matter: 45.7 calcium, 828.2 potassium, 215.8 magnesium, 722.7 phosphorous, 133.1 sodium, 5.4 iron, 12.5 zinc, 1.1 manganese, and 1.6 copper. Furthermore, yellow mealworm larvae are characterized to turn a wide range of organic waste into valuable protein, easy for farming and fast growth without increased use of resources like space and water or carbon emission excess (Rumpold and Schlüter, 2013; Ballitoc and Sun, 2013). Therefore, in the last decades, numerous companies have started the production of *T. molitor* for feed purposes to replace cereal-based feed partially or fully. In addition, several studies have evaluated the effects of yellow mealworm meal utilization on poultry growth performance (Ballitoc and Sun, 2013; Bovera et al. 2015; Bovera et al. 2016; Biasato et al. 2018). *T. molitor* farming is mainly achieved in humid Western where there is an abundant and wide range of substrates, including valuable recycled proteins from organic waste and by-products of agriculture and the food industries (Ramos-Elorduy et al. 2002). Dry climate regions such as the Middle East and

North Africa, including southern Algeria and Biskra, that are sparsely vegetated, with lower quantities of agricultural waste, discourage any temptation to raise yellow mealworm and to use it like poultry feed substitute. Moreover, most people in these arid and/or semi-arid regions have low in-comes and are not always able to pay for chicken meat derived from cereal nutrition. In these areas, there is less vegetable cultivation and consequently, there is less green waste; however, there are date palm (*Phoenix dactylifera* L., family: Palmaceae) plantations. This tree offers date fruit at the mature stage ("tamr" Arabic language) playing an important role in the autochthones day-to-day diet and economic and social lives (Gurevich et al. 2005). However, date palm fruit cultivation is often accompanied by enormous fruit losses which mainly occur during the date picking, date storage, conditioning and processing stages including date fruit seeds (pits) and date fruits that fall from the tree before maturity. According to non-published data, date-fruit waste could be estimated to an average of 20% of total date fruit production, corresponding to thousands of tons per year. Date-fruit waste is often discarded or used for limited purposes such as animal feed. Date-fruit waste is not edible for commercial purposes but represents the same nutritional values as the following commercialized date fruit chemical composition: 62% to 75% sugar, 2.2% to 2.7% protein, 0.4% to 0.7% fat, 5% to 8% fiber, 3.5% to 4.2% ash and vitamins, especially B-complex, C, and K (Abdel-Hafez et al. 1980; Baraem et al. 2006). Besides, the fruit pulp, date pit is about 10% of the fruit's weight and on average contained 2.3 to 6.4% protein, 5.0 to 13.2% fat, 3.1 to 7.1% moisture and 0.9 to 1.8% ash (Al-faarsi et al. 2007).

This study evaluates the growth performance of a new diet based on mixture of boiled-dried granulated *T. molitor* larvae (Tm) and date-fruit waste (Dw) in broiler chicken diet. This was done by (i) using date-fruit waste as food for *T. molitor* insect breeding to compensate for the lack of local vegetable waste and (ii) using the resultant insect larvae mixed with date-fruit waste as a partial alternative feed source in broilers meat production.

MATERIALS AND METHODS

The experimental protocol was designed in accordance to the guidelines of the Committee on Care and Use of Laboratory Animal Resources of the Algerian Association of Experimental Animal Sciences (AASEA); these guidelines are like those of the Guide for the Care and Use of Laboratory Animals.

Insects and substrate

Yellow mealworm, *Tenebrio molitor* larvae, (average weight 0.14 to 0.2 g per worm) were purchased from a fishing shop Fischereibedarf Niklaus, Berne (Switzerland). Larvae were reared in plastic containers (60 x 40 x 10 cm) with aeration slits in the side and maintained under climate-controlled chamber at 28 ± 2 °C, 45% to 65% relative humidity (RH) and a 24 hour dark photoperiod. To avoid unwanted contamination, rearing plastic containers were sanitized with active chlorine solution (3%) before use. Date-fruit waste, no matter what date varieties were procured from a local plantation in El Hadjeb, Biskra (Algeria) harvested from September 2018 to January 2019. Date-fruit waste (with date pits) was ridden of debris and milled through Corn Grinding Mill IndiaMart to pass through a 5 mm sieve, stored in airtight plastic bag at 4°C and designed in this work (Dw).

Larval growth and mass production of insects

Dw was used as diet for all yellow mealworm stages. For each container, 1 kg of Dw, a couple of vegetable strips (mainly carrot or potato), and mostly 500 *T. molitor* larvae were sampled. Larvae were allowed to feed ad libitum and based on visual observation of remaining diet and/or accumulated feces, the diet was refreshed. To provide moisture, vegetable strips were added twice a week and the old pieces were removed. Using 3% chlorine-washed sieve (3.35 mm openings), three to five months old larvae were collected directly from the feeding containers and starved for 24h before being killed by boiling in water for 3 minutes and then overnight oven-dried at 60 °C (Aguilar-Miranda et al. 2002). Dried yellow mealworm larvae were coarsely chopped using a Philips electric meat mincer and the resultant boiled-dried powdered *T. molitor* larvae (Tm) packed and stored at 4°C until further use. The Tm was examined for food poisoning pathogen contamination by assessing *Escherichia coli* and *Salmonella* spp. They were found to be safe from both cited pathogens.

Around 10% of total reared larvae per container were allowed to finish their whole development period (adult stage) in separated rearing containers. Larvae transform into pupae, and thence adult beetles emerge from the pupae stage. When reproducing, females usually lay eggs (typically produce 300 to 500 eggs at once) in meal containers. By sieving the bedding, larvae eggs mixed with substrate residues were sampled in new rearing container to maintain the future generation.

Experimented broilers feed formulation

During all studies, the broilers were fed exclusively with the followed diet formulations. Equal mixture of three commercialized cereal-based diets (Table 1). Chick starter feed, chick grower feed and chick finisher feed obtained from a local chicken food distributor in Biskra (Algeria) were formulated. The first control (C1) was 100% the commercial mixture. Second control (C2) was obtained by mixing 50% commercial mixture with 50% Dw. While the other groups (Diet1 and Diet2) were formulated by adding three ingredients at different proportions: 50% commercial mixture: 40% Dw: 10% Tm (Diet1) and 50% commercial mixture: 10% Dw: 40% Tm (Diet2) (Table 2). All experimental meal preparation of this study, including packing and storage, were carried out in ultra-clean conditions. The assessment of *Escherichia coli* and *Salmonella* spp. in all prepared meals formulation was not detectible.

Table 1. Ingredients of broiler meal used as first control (C1). This commercial mixture has resulted from equal mixture of three-broiler starter, grower, and finisher feed

Ingredients	Starter	Growth	Finisher
Corn meal	10.1	12	16.5
Wheat	50	50	50
Soybean meal 48 c.p	30	26	21.4
concentrated protein(1)	5	5	5
Sunflower oil	2.9	5.2	5.3
Calcium carbonate	0.9	0.9	0.9
Dicalcium phosphate	0.7	0.5	0.5
Sodium chloride	0.2	0.2	0.2
Mineral-vitamin-premix	0.2	0.2	0.2
Total	100	100	100
Calculated chemical analysis			
CP%	23	21.25	19.4
ME (kcal/kg)	3003	3153	3200
L-Lysine %	1.26	1.1	1.0
DL-Methionine %	0.48	0.45	0.43
Cysteine%	0.36	0.34	0.31
Methionine + Cysteine %	0.84	0.79	0.74
Arginine%	1.28	1.15	1
Ca %	0.85	0.80	0.78
Av. Phosphorus %	0.43	0.40	0.40

Note: (1) The concentrated protein type Brocon-5 special W contain the following per kg:20% crude protein, 5% fat, 2.2% fiber, 4.2% Ca, 4.68% P, 3.85% Lysine, 3.7% Methionine, 4.12% Methionine + Cysteine, 2.5% Na, 2107 ME (kcal.kg^{-1}), 2000 IU vitamin A, 4000 IU vitamin D_3, 500 mg vitamin E, 30 mg vitamin K_3, 15 mg vitamin B1, 140 mg vitamin B_2, 20 mg B_6, 10 mg Folic acid, 100 µg Biotin, 1 mg Fe, 100 mg Cu, 1.2 mg Mn, 800 mg Zn, 15 mg I, 2 mg Se, 6 mg Co, 900 mg Antioxidant.

Table 2. Composition of experimental diets

Ingredients	Diet formulation			
	C1	C2	Diet1	Diet2
Commercial mixture	100%	50%	50%	50%
Dw	-	50%	40%	10%
Tm	-	-	10%	40%

The commercial mixture resulted from equal mixture of three-broiler starter, grower, and finisher feed. Dw, Date-fruit waste; Tm, powdered *T. molitor* larvae.

Broilers feed comparison

This study was performed using Ross-308 male and female broiler chicks purchased from a local poultry corporation named CFPA Amairi Aissa located in Manbaa el-Ghozlane/Loutaya (Algeria) at one-day of age. Healthy broilers were selected for the study after being acclimatized to the lab overnight. A total of 56 two-day age broilers averaging 45.3 ± 0.9 g were randomly allotted to 4 dietary groups each with 2 replicates consisting of 7 chickens (C1, C2, Diet1, and Diet2). Each group was housed in enclosure of 1.50 m wide x 2.20 m long x 1.10 m high and was equipped with poultry drinker with capacity 3 liters, sawdust as litter, and feeder filled with the specific diet formulation for every corresponding group. Based on standard breeding practices (Aviagen, 2014), during the first 2 weeks, chicken house was heated by infrared lamps to maintain the suitable temperature of 33°C then gradually reduced according to the age of broilers (3°C every week) until reaching 21°C and then kept constant. Until day 6, chicken houses were illuminated with 22:2 light-dark cycle and then 18:6 light-dark cycle until slaughter age. Broilers were kept under similar managerial and environmental conditions with free access to water and feed. At hatching, all broiler chicks were vaccinated against Newcastle disease, Gumboro disease, infectious bronchitis, and coccidiosis and vaccine recalls were performed on day 9 for infectious bronchitis and on day 18 for Gumboro and Newcastle diseases.

Feed and water intake, individual live body weight and mortality rate were recorded daily in the morning and evening for each group, starting at day 2 until the last day of the experiment day 60. The feeding trial lasted for 8 weeks. After measurement of body weight, clinical signs rate and blood sampling, all animals were slaughtered and immediately digestive systems were recovered and processed for bacteriological and copro-parasitological analysis. All body and feed weight measurements were monitored using a precision electronic scale OHAUS Scout SE.

Hematological and biochemical parameters

At slaughter and under safe handling practices, blood of four chickens per feeding group was collected by puncturing the medial wing vein using a syringe (26-gauge x 13 mm needle). From each chicken, 1 ml blood was placed in EDTA anticoagulated tube and 1 ml in Eppendorf tube without anticoagulant. On one glass slide, unfixed blood smears were prepared, air-dried and stained in concentrated May-Grunwald stain 6 min, 1:1 May-Grunwald stain-distilled water for 90 sec and 1:9 Giemsa's stain for 15 min (Robertson and Maxwell 1990). Using Neubauer hemocytometer, the total red and white blood cell counts were determined on blood samples previously treated with a 1:200 Natt-Herrick solution. 100 leukocytes per slide, including granular (heterophils, eosinophils, and basophils) and non-granular (lymphocytes and monocytes) leukocytes, were counted and the complete blood count results are presented as the percentage of each cell occurring in each stream. The heterophils to lymphocytes ration (H/L) was calculated by dividing the number of heterophils by the number of lymphocytes (Gross and Siegel, 1983). For biochemical analysis, the tubes without anticoagulant were left in vertical position at room temperature for approximately two hours, then centrifuged at 800g/10 min/4°C. Sera were sampled in new Eppendorf and frozen at -20°C until analysis. The serum concentrations of total protein, albumin, glucose, cholesterol, triglycerides, total bilirubin, creatinine, uric acid, iron, total calcium, phosphorus, magnesium, and activities of the enzymes aspartate-aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), Alkaline phosphatase (ALP) were measured by means of enzymatic methods in a clinical laboratory analysis Biskra, Algeria.

Copro-parasitological and bacteriological examination

To investigate fecal egg (nematode, cestode) and fecal coccidial oocysts, the cloacae of the same slaughtered broilers chosen for blood analysis (identified with a shank ring) were cut open and processed using supersaturated saline flotation technique as modified McMaster method (Ballweber et al. 2014). Feces were homogenized in 15 ml of water in plastic bottle, filtrated through fine-mesh sieve (aperture 150 µm) and centrifuged at 1500 rpm for 2 minutes. Resulting sediment was suspended in 10 ml of the floatation medium (400 g of NaCl in 1000 ml of distilled water). A coverslip was placed gently on the test tube and allowed to stand on a level surface for at least 15-20 min. The coverslip was carefully removed and placed on a glass slide and examined immediately for intestinal eggs under x10 and x40 objective lens. Examination was aided by the addition of Lugol's Iodine solution to the sample on the glass slide. For bacteriological investigation, inoculum from the gastrointestinal tract (esophagus, crop, gizzard, colon, and cloacae) were made on various selective and differential media for bacteria isolation. All inoculated media were incubated at 37°C for 24-48 h. For enrichment, samples collected for salmonella isolation were inoculated in Selenite-Cystine Broth and incubated at 37°C for 24 h followed by inoculation on MacConkey agar. All bacteriological culture media are commercially available (BioRad) as pre-made or as bases, which can be prepared according to the manufacturer's recommendations. After 24-48 h of incubation at 37°C, colonies were examined for cultural and morphological properties on growth media.

Thereafter, smears on slides were prepared from the colonies for gram staining to classify the isolates into Gram-positive or Gram-negative under light microscopy at x100 magnification under oil immersion. The Gram stain procedure was performed following the protocol described in a standard microbiology laboratory manual (Leboffe and Pierce, 2002). For species identification, pure colonies obtained from subculture were subjected to standard biochemical tests as described elsewhere (Holt et al. 1994).

Statistical analysis

NCSS 2019, version 19.0.2 was used for data analysis. Data collected for growth performance and serum components were tested by one-way ANOVA, using analysis of variances followed by Tukey's multiple comparison test to evaluate dietary Dw and/or Dw-Tm inclusion. *P* values <0.05 were considered statically significant. Results were expressed as mean and standard deviation (SD).

RESULTS AND DISCUSSION

Insect

Yellow mealworms were allowed to feed ad libitum Dw supplemented with vegetable strip until larvae stage started pupating, which can occur after 3 to 5 months. At this time, pupae (pupa is a free-living creature) were sexed based on their morphology. Almost 50 pupae, 35 were females and 15 were males with sex identified by structural differences in the 4th and 5th visible abdominal sternites (Bhattacharya et al. 1970). Pupae were carefully removed and placed in new containers containing 1 kg of Dw and vegetable strips to allow adults to lay eggs which happened 4 to 17 days after copulation.

This maintaining step is very important to ensure the breeding of future generations who are kept under similar managerial and environmental conditions. Once pupae were kept separated, all yellow mealworms supposed at last instar larvae were removed into collector container to be processed as was mentioned before. Insect development time and mortality rate until pupation were similar in all larvae batches whose weight at the later larval stage was about 0.17 ± 0.3 g for 25 to 35 mm long. No difference in number of days from pupation and the corresponding pupae size (12 to 18 mm long) until the pupae metamorphosis into beetles was observed throughout the study period.

Growth performance

On study feeding start day, average live body weight of all birds regardless of related diet group was 45.3 ± 0.9 g. From this day (day two of age) until the end of experiment, all group's birds showed an increasing body weight. However, group C2 broilers showed greater growth depression versus all other dietary groups. The growth weight of broilers recorded in group Diet2 was significantly higher (*P*<0.05) than two broilers groups fed respectively with the mixture of commercialized cereal-based diets C1 and Diet1 (Figure 1), which suggested that DW and Tm mixture (Dw-Tm) are an effective ingredient for broiler feed that increases the live body weight. Bodyweight (BW) (Table 3) of broilers in group Diet2 (2601.69±168.89) was significantly higher (*P*<0.05) than C1 and Diet1. The lowest BW was noted in C2 (1458.95±239.03).

C1 = mixture of three commercialized cereal-based diets; C2 = 50% C1 + 50% Dw; Diet1 = 50% C1 + 40% Dw + 10% Tm; Diet2 = 50% C1 + 10% Dw + 40% Tm. (*) C1 is significantly higher (*P*<0.05) than C2, (**) Diet2 is significantly higher (*P*<0.05) than Diet1 and significantly higher (*P*<0.05) than C1 (***), with statistical significance: (*P*<0.05).

Feed intake of broilers (Table 3) in group Diet2 was higher (6035.42±215.89) than birds in other groups. The lowest feed intake (5019.02±203,67) was recorded in broilers group C2. The feed intake differed significantly higher (*P*<0.05) when broilers in groups Diet1 and Diet2 were compared with broilers of groups C1 and C2. The results further suggested that inclusion of Dw-Tm in broilers diet could be adequately used as an ingredient for broilers feed to get higher body weight and growth weight over commonly used as commercial broiler feed. It can be noted that live body weight increased with increasing level of Tm.

The difference improvement of the FCR efficiency was not so pronounced when the broilers were fed either with commercial diet (C1) or with diet containing Dw-Tm. The FCR was statically non-significant (P>0.05). The significantly lowest (P<0.05) FCR in all other dietary groups was calculated in broilers of group C2. The results showed essentially similar feed conversion efficiency in C1 (2.35±0.05), Diet1 (2.49±0.09) and Diet2 (2.32±0,07) which suggested that Dw could be substitute for almost half of commercial diet but only when is associated with Tm. Diet2 (C150%:0% Dw: 40% Tm) presents relatively poor FCR compared with Diet1 (C150%: 40% Dw: 10% Tm) this suggested that increasing the level of Tm to 40% in broilers feed caused improving the FCR.

Hematological and biochemical parameters

No significant (P>0.05) differences were observed among values in hematological and serum parameters when comparing all feeding groups (Table 4).

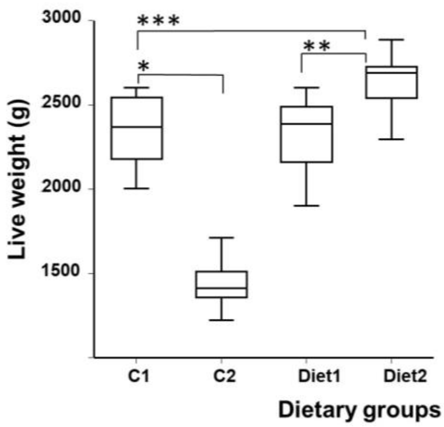

Figure 1. Live weight body of the dietary groups at slaughter day

Table 3. The growth performance of broilers as influenced by feeding various dietary regimes

Variables	Dietary groups			
	C1	C2	Diet1	Diet2
BW (g)	2358.40±140.67^{a}	1458.95±239.03^{b}	2332.84±202.47^{a}	2601.69±168.89^{c}
CFI (g)	5548.71±195.83^{a}	5019.02±203.67^{a}	5808.08±200.47^{b}	6035.42±215.89^{b}
FCR (g/g)	2.35±0.05^{a}	3.44±0.08^{b}	2.49±0.09^{a}	2.32±0.07^{a}

Note: ^{abc} Means within each row with no common superscript differ significantly (P<0,05) (ANOVA). C1 = mixture of three commercialized cereal-based diets; C2 = 50% C1 + 50% Dw; Diet1 = 50% C1 + 40% Dw + 10% Tm; Diet2 = 50% C1 + 10% Dw + 40% Tm. CFI = cumulative feeding intake; BW = Body weight; FCR = feed conversion ratio. Variables were given in mean ± standard deviation.

Table 4. The effect of partial substitution of commercial diet by Dw and Tm inclusion on the hematological and serum parameters in broilers.

Parameters	Dietary groups			
	C1	C2	Diet1	Diet2
Eryt. (10^6 cell.µL^{-1})	2.4±0.39	2.1±0.44	2.5±0.31	2.1±0..56
Leuk. (10^3 cell.µL^{-1})	7.89±0.49	8.14±0.53	7.63±0.41	7.75±0.42
H/L ratio	0.89±0.02	0.92±0.04	0.87±0.05	0.91±0.02
Lymphocyte (%)	57.18±5.23	52.18±7.02	49.18±6.15	55.35±9.03
Heterophil (%)	38.3±4.11	32.5±7.65	33.9±6.21	37.7±6.82
Monocyte (%)	3.40±1.09	3.00±1.14	3.20±1.2	3.50±1.10
Basophil (%)	2.1±0.52	1.7±0.60	2.2±0.12	1.9±0.47
Eosinophil (%)	3.38±1.49	3.04±1.05	3.40±1.01	2.08±1.14
Total protein (g.dL^{-1})	4.21±0.40	3.65±0.72	4.05±0.25	4.25±0.65
Albumin (g.dL^{-1})	2.23±0.09	1.83±0.08	1.92±0.05	2.31±0.07
Glucose (mg.dL^{-1})	271.2±30,07	261.2±60.0	252.8±28.12	241.9±39.20
Trigly. (mgdL^{-1})	68.18±20,81	74.18±40.3	78.54±36.55	89.51±41.36
Cholesterol (mg.dL^{-1})	122.25±5.14	118.25±7.4	122.26±8.14	125.12±6.03
Total bilir. (mg.dL^{-1})	0.40±0.12	0.38±0.18	0.40±0.20	0.39±0.13
Direct bilir. (mg.dL^{-1})	0.16±0.07	0.09±0.01	0.06±0.05	0.67±0.04
Uric acid (mg.dL^{-1})	6.05±0.71	5.14±0.80	8.11±0.28	8.05±0.501
Creatinine (mg.dL^{-1})	0.32±0.05	0.40±0.07	0.37±0,08	0.35±0.04
Total calcium (mg.dL^{-1})	10.15±0.76	09.56±0.46	10.15±0.76	11.04±0.49
Phosphorus (mg.dL^{-1})	6.58±0,62	6.14±0.42	6.28±0.38	6.50±0.41
Magnesium (mg.dL^{-1})	2.31±0.53	2.11±0.41	2.28±0.41	2.20±0.31
Iron (µg.dL^{-1})	100.52±8.52	79.52±10.81	95,74±4,55	91,52±8,22
AST (UI.mL^{-1})	251.25±19.30	181.25±22.14	201.25±40.26	262.25±50.11
ALT (UI.L^{-1})	9.10±2.01	9.88±5.38	10.12±5.19	11.02±3.36
GGT (UI.L^{-1})	29.17±7.80	21.17±9.62	30.17±8.12	22.17±6.41
ALP (UI.L^{-1})	12.45±9.06	15.32±8.10	17.05±8.44	18.23±10.27

Each mean represents two replicates with 3 broilers/replicate. C1 = mixture of three commercialized cereal-based diets; C2 = 50% C1 + 50% Dw; Diet1 = 50% C1 + 40% Dw + 10% Tm; Diet2 = 50% C1 + 10% Dw + 40% Tm. Eryt., erythrocyte; Leuk., leukocyte; H/L, heterophils to lymphocytes ration; bilir., bilirubinr; AST, aspartate-aminotransferase; ALT, alaninoaminotransferase; GGT, gamma glutamyl transferase; ALP, Alkaline phosphatase. Parameters were given in mean ± standard deviation

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Production and effect of vermiwash singly and in combination with vermicompost on the growth, development, and productivity of tomatoes in the greenhouse in Suriname

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Abstract. *Awadhpersad VRR, Ori L, Ansari A. 2021. Production and effect of vermiwash singly and in combination with vermicompost on the growth, development, and productivity of tomatoes in the greenhouse in Suriname. Asian J Agric 5: 29-34.* In Suriname farmers often largely rely on high inputs of synthetic fertilizers and pesticides to achieve high yield. To overcome this, sustainable agriculture seeks to introduce agricultural practices that are environmentally sound, economically viable, and socially supportive. In the present study, the effect of vermicompost and vermiwash and in combination was evaluated on the growth and yield of tomato (*Lycopersicon esculentum* Mill.) in the greenhouse. The experiment was a Randomized Block Design with four treatments and three replications. The growth parameters were measured for plant height, shoot wet and dry weight, root weight, and length, and yield in terms of the number of fruits and fruit weight. The produced vermiwash was a brownish colored liquid and had all the essential macro and micro plant nutrients, which indicates an environmentally friendly enriched nutrient liquid fertilizer for sustainable agriculture. The research results at harvest time indicated that the plant height, shoot fresh and dry weight, root weight, root density, root length, yield and fruit weight were higher for the plants treated with a combination of vermicompost and vermiwash. It was also noted that the flowering and fruiting ratio were significantly enhanced by application of vermiwash as a foliar spray. The combination of vermicompost and vermiwash (50 g + 50 mL) significantly ($p < 0.05$) resulted in the highest yielding plants, followed by vermiwash (100 mL) and vermicompost (100 g).

Keywords: Biofertilizer, earthworms, organic tomato, vermicompost, vermiwash

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) belongs to the Solanaceae family and is a popular vegetable widely grown in the tropics, including Suriname. According to the Ministry of Agriculture, Animal Husbandry and Fisheries (LVV) statistical data, the total tomato production area in 2017 was approximately 119 ha. with a total yield of 1.442 tons of tomato fruits, which makes tomato one of the most cultivated crops in Suriname. This crop is an excellent source of minerals and vitamins, including iron, phosphorus, vitamin A and C (Bhowmik et al. 2012).

In Suriname, agricultural practices largely rely on high inputs of synthetic fertilizers and pesticides to achieve high yield and protect the crops against pathogens and pests. Excessive use of fertilizers and pesticides leads to gradual degradation of soil fertility and microbiological diversity (Samadhiya et al. 2013). This decline in soil quality further leads to water and land pollution, thereby lowering the land's worth.

The massive application of pesticides and synthetic fertilizers also leads to high residue levels in crops, which is bad for human health. Nowadays, consumers are more aware of the food they consume and the chemicals that are used for crop production. Therefore, it is very important to seek alternative bio-fertilizers as a supplement for chemical fertilizers.

Presently, there is a strong interest in alternative strategies to ensure competitive yields, protection of crops, environment, and the health of humankind. Sustainable agriculture seeks to introduce agricultural practices that are environmentally sound, economically viable, and socially supportive. In this context, alternative sources such as microbial inoculants and composted products are considered to meet the nutrient requirements of crops.

Earthworms are known to decompose organic waste into nutrient-rich vermicasts through the combined action with microorganisms. The produced vermicompost is reported to be rich in nitrogen (N), phosphorus (P), potassium (K) and micronutrients, with a greater rate of microbial and enzymatic activities. Several researchers found that vermicompost has a positive effect on the growth, development, flowering, and yield of plants. It has also been noted that vermicompost increases the root apparatus and the biomass production of the plants and improves the soil fertility (Manyuchi 2016; Zaefarian and Rezvani 2016).

The by-product of the vermicomposting process, which is termed vermiwash is a brownish colored substance that is formed due to the movement of water in the vermicomposting units through the burrows formed by the earthworms. This liquid is reported to be rich in NPK components, micronutrients, plant growth hormones, microbes, and enzymes. It is used as a foliar spray that plants can easily absorb (Manyuchi et al. 2013; Kaur et al. 2015). The foliar application of vermiwash is also reported to have a pesticide effect, with plants showing less or no incidence of diseases and pests (Verma et al. 2018).

Both vermicompost and vermiwash are used as bio-fertilizers in sustainable agriculture practices. It is reported that the combined use of vermiwash and vermicompost leads to the highest yielding plants with more branches, higher number of capsules, higher plant dry weight, improve root growth parameters, improve physicochemical, biological, and microbiological properties of the soil (Makker et al. 2017). Improving the growing conditions with vermiwash and vermicompost enhance the quality of the crop, by increasing their nutrition status, which also improves the sustainability of commercial agriculture in a less tangible, but equally important way, since the main goal of agriculture is to grow food for the well-being of the population. Therefore, this research aims to provide an insight on how to produce vermiwash out of organic waste material and its effect on crop production singly and in combination with vermicompost.

MATERIALS AND METHODS

Production of vermiwash

The research was conducted in the vermicomposting station at the Anton de Kom University of Suriname at the Leysweg, Paramaribo. The experiment was set up in 9 plastic buckets. The *Eisenia foetida* earthworms were collected from the existing vermicomposting station.

The Vermiwash culture bed was prepared in plastic bucket of 20 liters and a tap was fixed at the lower side of each bucket to regulate the water supply. The basal layer consisted of pebbles (4.5 cm) and a layer of coarse sand (4.5 cm) to ensure proper drainage. On top of the basal layer a layer of loamy soil (8 cm) was placed and moistened. Approximately 50 earthworms were introduced per bucket of different age groups of juveniles, non-clittelate and clittelate earthworms. On top of the soil fresh/dry cattle dung was scattered (4.5 cm) and a combination of dry grass clippings and dry neem leaves was added.

Approximately after 60 days of vermicomposting the tap was closed. On top of the bucket, a water sprinkler was hung. About 1 liter of water (the volume of water is 1/20 of the size of the bucket) was poured into the sprinkler and allowed to gradually sprinkle on the bucket overnight. The tap was opened the next day to collect the Vermiwash.

The physicochemical analysis was conducted for the obtained vermiwash, the vermicompost of rice straw collected from the existing vermicompost unit, and the initial and final soil samples using the methods described according to the soil laboratory prescriptions of the Anton de Kom University of Suriname.

Cultivation of tomato plants

Experimental site

The research was conducted at the Anton de Kom University of Suriname at the Leysweg, Paramaribo, with latitude/longitude: 5° 48' 29.99" N/55° 12' 35.40" W and with an altitude of 3 m above sea level. The mean maximum and minimum day temperatures (C) in the greenhouse were 30.61 & 29.50 respectively. Relative humidity ranged from 70.97 - 77.81%.

Experimental layout/set-up

The experiment was set up in the greenhouse in pots from March to August 2018. The experiment was a Randomized Block Design (RBD) with 4 treatments and 3 replications. Each block consisted of four rows and seven plants per row. The treatments are given in Table 1.

For the implementation of the experiment three-week old tomato seedlings of the variety Delhi 501 were used. These were transplanted in earthen plant pots of a volume of 12 liters. Before transplanting, the earthen plant pots were irrigated, and plant holes were made. Initially, the amount of fertilizer was added, according to the treatments in the plant holes, after which the tomato plants were transplanted. Afterward, according to the treatments, the tomato plants were fertilized at an interval of two weeks, and vermiwash was used as a foliar spray. In total, during the cultivation period, the tomato plants were four times fertilized. The total amount of fertilizer added per plant is given in Table 2.

During the cultivation period of the tomato plants once a week the plant height was measured. At the end of the experiment, the root length was measured, and the biomass was determined, with wet and dry weight of the shoots and roots. For the production, the number and weight of fruits per plant were recorded. Data were pooled from all replications to calculate the average yield per plant.

Table 1. Treatments and their added quantities per plant

Treatment	Quantity added per plant
Control	No additives
Vermicompost	100 g
Vermiwash	100 mL
Combination of vermicompost and vermiwash	50 g + 50 mL

Table 2. Total amount of fertilizer added per plant

Treatment	Total amount added per plant
Control	No additives
Vermicompost	400 g
Vermiwash	400 mL
Combination of vermicompost and vermiwash	200 g + 200 mL

RESULTS AND DISCUSSION

Physicochemical properties of vermiwash

The harvested vermiwash from the beginning till day 60, showed a color change in the liquid from transparent to light yellow to brown, where the maximum nutrient value of the vermiwash was found.

The physicochemical properties of the vermiwash indicated the presence of nutrients in a significant quantity, which is also confirmed by Ansari and Sukhraj (2010); Kaur et al. (2015). The results of the physicochemical properties are shown in Table 3 and agree with the work done by Ansari and Sukhraj (2010). Although it had to be noted that several researchers found different nutritional value for the vermiwash, because the nutritional value depends on the feed used for the vermicomposting process and quality of the vermicompost (Kaur et al. 2015; Zaefarian and Rezvani 2016).

Physicochemical properties of the soil

The physicochemical properties of the soil at the beginning and at the end of the experiment are shown in Table 4. The soil analysis at the beginning of the experiment showed that the pH was alkaline; this result was obtained due to the fact that for the soil analysis the sample was ground fine and so the shells in the sample, were the cause that the result was alkaline. Measurement of the sample with the soil pH meter showed that the pH was almost natural. The physicochemical properties of the soil were acceptable for the cultivation of the tomato plants.

At the end of the experiment, a mixed sample was taken from each treatment to determine the nutrient values. Comparison of the soil nutrient at the beginning and at the end of the experiment showed that there was no difference (Table 4). At the end of the experiment, the nutrient values of the treated soils for Exchangeable P, K, Ca, and Mg were slightly higher than the nutrient value of the soil at the beginning of the experiment. The overall highest value is seen for the combination treatment.

As reported by researchers, the combination of vermicompost and vermiwash has a positive effect on the biochemical characteristics of the soil, there are marked improvements in soil micronutrients, physical and chemical properties (Ansari and Sukhraj 2010; Tharmaraj et al. 2011).

It is also reported that vermicompost has enzymes that break down the organic matter in the soil to release the nutrients, so it rejuvenates the depleted soil fertility, increases the water holding capacity, maintains the soil quality, and enriches the nutrient composition (Adhikary 2012; Prabina et al. 2018).

Vegetative growth

Plant height

The results obtained showed that all the plants are grown on vermicompost and vermiwash singly and in combination had a significant shoot growth (Table 5). The tallest plants were observed for the plants fertilized with a combination of vermicompost and vermiwash (112.62 +/- 4.33 cm), which also had the maximum (98.69 cm) increase in height. The control plants showed minimum shoot growth because the nutrients were not available in sufficient quantity. The results of the LSD test showed that there was a significant difference between the treated and the control plants ($p < 0.05$) and between the vermicompost and combination plants ($p = 0.005$). There was no significant difference between the vermicompost and vermiwash plants ($p = 0.175$) and the vermiwash and combination plants ($p = 0.148$).

Vermicompost and vermiwash are reported to be rich in nitrogen (N), phosphorus (P), potassium (K), and micronutrients, with a greater rate of microbial and enzymatic activities (Manyuchi et al. 2013; Zaefarian and Rezvani 2016). They also contain hormones like auxins, cytokinesis, gibberellins, and humic acids, which are responsible for plant growth. It is also reported that vermicompost and vermiwash are enriched in certain metabolites and vitamins that belong to the B group and provitamin D which help to enhance plant growth (Jaikisun et al. 2014; Lujan-Hdalgo and Celina 2016).

Table 3. Physicochemical properties of vermiwash harvested at day 60

Parameters	Vermiwash
pH H2O	7.30
EC (mS)	8.93
Tot. N (ppm)	216.00
Tot. P (ppm)	70.00
Tot. K (ppm)	1327.63
Tot. Ca (ppm)	258.17
Tot. Mg (ppm)	210.54
Tot. Na (ppm)	245.16

Table 4. Physicochemical properties of the soil at the beginning and at the end of the experiment

Parameters	Begin	End of the experiment			
		C	V	W	VW
pH H2O	8.10	8.30	8.00	7.90	7.90
EC (mS)	2.40	2.13	3.02	2.63	3.08
CEC (meq/100 g)	8.48	10.40	8.89	9.30	9.45
Org. C (%)	4.29	4.21	3.66	4.46	3.87
Tot. N (%)	0.24	0.18	0.22	0.19	0.21
Tot. P (%)	0.01	0.02	0.03	0.04	0.04
Tot. K (%)	0.05	0.07	0.07	0.09	0.09
Tot Ca (%)	6.21	7.55	7.38	8.82	9.00
Tot. Mg (%)	0.18	0.18	0.17	0.19	0.21
Tot. Na (%)	0.36	0.31	0.38	0.41	0.42

Note: Treatment codes; C: Control; V: Vermicompost; W: Vermiwash; VW: Vermicompost + Vermiwash

Table 5. Plant height (Mean ± SEM) and % increase

Week	Treatments			
	C	**V**	**W**	**VW**
1	11.40 ± 1.87	12.71 ± 1.76	12.79 ± 1.70	13.93 ± 1.33
2	13.42 ± 2.11	20.88 ± 2.45	17.48 ± 2.21	22.93 ± 1.62
3	19.69 ± 2.73	29.40 ± 6.76	29.26 ± 5.19	36.21 ± 3.62
4	26.17 ± 3.88	47.48 ± 9.70	46.10 ± 7.93	54.67 ± 4.32
5	30.57 ± 6.27	57.59 ± 9.72	58.17 ± 8.05	66.67 ± 6.42
6	41.36 ± 7.89	69.69 ± 5.25	78.62 ± 6.63	82.90 ± 3.52
7	53.81 ± 8.03	80.80 ± 8.19	89.29 ± 7.58	92.52 ± 4.07
8	67.38 ± 6.87	89.14 ± 9.54	98.95 ± 7.74	100.00 ± 7.31
9	79.62 ± 6.79	94.43 ± 11.90	106.33 ± 8.64	108.90 ± 5.51
10	85.38 ± 7.37	100.90 ± 11.69	108.81 ± 11.16	112.62 ± 4.33
Increase (cm)	73.98	88.19	96.02	98.69
Increase (%)	87	87	88	88
Ranking	a	b	bc	c

Note: The different letters of the ranking are significantly different at P≤0.05 according to LSD multiple range test. Treatment code: C: Control; V: Vermicompost; W: Vermiwash; VW: Vermicompost + Vermiwash

Shoot fresh and dry weight

The LSD test for shoot fresh and dry weight showed that there was a significant difference between the treatments (p= 0.000). The highest average shoot fresh and dry weight between the treatments were recorded for the plants fertilized with vermiwash (W) (resp. 1107 ± 0.45 g, 320 ± 0.40 g) and the lowest for the control plants (C) (resp. 160 ± 4.04 g, 83 ± 0.21 g). The moisture content was the highest for the plants fertilized with vermicompost (810 g) and the lowest for the control plants (77 g) (Table 6), which means that the plants fertilized with vermicompost had more moisture in their tissue. This result is in line with the fact that vermicompost has a moisture content of about 32 - 66% and is humus like sweet-smelling compost material (Adhikary 2012).

According to a study on plant biomass of strawberries, addition of vermicompost increased the plant dry weight (Joshi and Vig 2010). As for the addition of vermiwash, it has been reported that it exhibited growth-promoting effects on the exo-morphological characters such as plant height, length and diameter of the internode, number of leaves, leaf surface area and wet and dry weight of the shoot (Samadhiya et al. 2013; Kaur et al. 2015). A study about the effect of vermiwash on the growth parameters of brinjal plants found that the results from the vermiwash were a little bit higher compared to the vermicompost which is also seen in this research (Jaybhaye et al. 2015).

Another study reported that the combination of vermiwash and vermicompost resulted in the highest plant dry weight (Makker et al. 2017).

Root fresh and dry weight and root length

The highest root fresh and dry weight was measured for the combination treatment and the lowest for the control plants, with respectively the maximum moisture content of 123.33 and the minimum of 3.33. The results of the LSD test for the root dry weight showed that there was a significant difference between all the treatments ($p < 0.05$) (Table 7).

The plants treated with the combination of vermicompost and vermiwash had the longest roots (97.67 ± 5.51 cm), followed by the plants treated with vermiwash (91.33 ± 8.08 cm), vermicompost (78.33 ± 14.01 cm), and the control plants (38.67 ± 1.53 cm). The LSD test also showed that there was a significant difference between the treated and control plants (p= 0.000) and between the plants treated with vermicompost and a combination of vermicompost and vermiwash (p= 0.101) (Table 8.).

According to Tomati et al. in 1988, earthworm casts promote root initiation, root biomass and root percentage. It is also reported that vermicompost has a positive effect on plant development and promotes root length (Jaikisun et al. 2014). Studies also suggested that the use of vermicompost and vermiwash separately increase the wet and dry weight of roots and root length, and the combination of vermicompost and vermiwash have much better results (Samadhiya et al. 2013; Sundrarasu and Jeyasankar 2014; Kaur et al. 2015; Makker et al. 2017). The effect of vermiwash and vermicompost on the enhanced root growth parameters can be attributed to the presence of humic and fulvic acids. These compounds have been shown to increase plant height, dry and fresh weight of plants and roots as well as enhance nutrient uptake by increasing the root cell membrane permeability (Wright and Lenssen 2013; Makker et al. 2017).

Table 6. Shoot fresh – and dry weight (Mean ± SEM) in grams and moisture content (%)

Treatment	Shoot fresh weight (Mean ± SEM)	Shoot dry weight (Mean ± SEM)	Moisture content (%)
C	160 ± 4.04 a	83 ± 0.21 a	77
V	1030 ± 0.80 b	220 ± 0.26 b	810
W	1107 ± 0.45 c	320 ± 0.40 c	787
VW	1070 ± 0.70 d	286 ± 0.25 d	784

Note: Values followed by different letters are significantly different at P≤0.05 according to LSD multiple range test. Treatment code: C: Control; V: Vermicompost; W: Vermiwash; VW: Vermicompost + Vermiwash

Table 7. Root fresh – and dry weight (Mean ± SEM) in grams and % moisture content

Treatment	Root fresh weight (Mean ± SEM)	Root dry weight (Mean ± SEM)	Moisture content (%)
C	8.00 ± 1.73 a	4.67 ± 1.53 a	3.33
V	110 ± 17.32 b	46.67 ± 7.63 b	63.33
W	84.33 ± 3.79 b	19.33 ± 1.15 c	65
VW	213.33 ± 77.67 c	90.00 ± 10.00 d	123.33

Note: Values followed by different letters are significantly different at P≤0.05 according to LSD multiple range test. Treatment code: C: Control; V: Vermicompost; W: Vermiwash; VW: Vermicompost + Vermiwash

Table 8. Average root length (Mean ± SEM)

Treatment	Root length (Mean ± SEM)
C	38.67 ± 1.53 a
V	78.33 ± 14.01 b
W	91.33 ± 8.08 bc
VW	97.67 ± 5.51 c

Note: Values followed by different letters are significantly different at P≤0.05 according to LSD multiple range test. Treatment code:C: Control; V: Vermicompost; W: Vermiwash; VW: Vermicompost + Vermiwash

Table 9. Average number of fruits – and fruit weight per plant (Mean ± SEM)

Treatment	Number of fruits per plant (Mean ± SEM)	Fruit weight per plant (Mean ± SEM)
C	-	-
V	25.43 ± 3.61 a	1295.34 ± 183.67 a
W	32.86 ± 2.86 b	1673.51 ± 145.52 b
VW	38.81 ± 0.41 c	1919.88 ± 20.40 c

Note: Values followed by different letters are significantly different at P≤0.05 according to LSD multiple range test. Treatment code: C: Control; V: Vermicompost; W: Vermiwash; VW: Vermicompost + Vermiwash

During this research, vermiwash was used as a foliar spray and not applied to the roots. In comparison to the control plants, vermiwash plants had a bigger and longer root system, which is caused by the available nutrients, hormones, and enzymes present in the vermiwash. This could also be the reason why the roots of the vermicompost treatment were bigger than the roots of the vermiwash treatment. The enhanced results of the combined effect of vermicompost and vermiwash are shown in the combination treatment of vermicompost and vermiwash, where the root structure was the biggest and the roots the longest.

Production of tomato plants

The yield results indicated that the treatments significantly differed from each other ($p<0.05$). The highest average yield (16.52 ± 1.01) and fruit weight (646.71 ± 68.09 g) per plant were recorded for the plants treated with the combination of vermicompost and vermiwash and the lowest average yield (9.38 ± 0.44) and fruit weight (380.52 ± 31.88 g) per plant for the plants treated with vermicompost only (Table 9). There has also been observed that the plants treated with the combination of vermicompost and vermiwash induced early flowering.

The results of the research at harvest time indicated that the plant height, shoot fresh and dry weight root weight, root density, root length, yield and fruit weight were higher for the plants treated with a combination of vermicompost and vermiwash. It was also noted that the flowering and fruiting ratio were significantly enhanced by application of vermiwash as a foliar spray, which was in line with the research done by Makker et al. in 2017. The results also showed that when vermicompost and vermiwash were used separately, they had a positive effect on plant growth, development, and yield. Studies revealed that the application of vermicompost and vermiwash separately and in combination enhance the plant growth parameters (plant height, stem thickness, and number of leaves) and yield parameters (number of flowers, fruits per plant and fruit weight) (Jaybhaye et al. 2015; Kaur et al. 2015; Maheswari et al. 2016; Makker et al. 2017).

It is also stated that foliar application of vermiwash shortens the life cycle of flowering and fruiting plants and that the fruits obtained from the combination of vermiwash and vermicompost showed even and uniform ripening (Makker et al. 2017). It is suggested that uniform maturing and fruit ripening is achieved with foliar spray of vermiwash (Makker et al. 2017).

Research investigators also stated that the flowering and fruiting ratio significantly increased for the plants treated with a combination of vermicompost and vermiwash (Sundrarasu and Jeyasankar 2014; Maheswari et al. 2016).

In summary, from this research, it can be concluded that the produced vermiwash was a brownish-colored liquid. It had all the essential macro and micro plant nutrients like N, P, K, Ca, Mg and Na which indicated the achievement of an environmentally friendly enriched nutrient liquid fertilizer. Vermicompost, vermiwash, and the combination of vermicompost and vermiwash as a biofertilizer had a positive effect on the plant growth parameters and yield parameters. The combination of vermicompost and vermiwash resulted in early reproduction and the highest yielding plants. Furthermore, the analysis of the soil samples before and after harvesting indicated a slight difference of elements in the soil. The combination of vermicompost and vermiwash notably enriched the soil with plant-available P and K elements.

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Genetic diversity of weedy rice (*Oryza sativa* f. *spontanea*) populations in Sri Lanka: An application of Self Organizing Map (SOM)

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Abstract. *Weerakoon SR, Somaratne S. 2021. Genetic diversity of weedy rice (*Oryza sativa *f.* spontanea*) populations in Sri Lanka: An application of Self Organizing Map (SOM). Asian J Agric 5: 35-43.* Weedy rice (WR) (*Oryza sativa* f. s*pontanea*) has become a major threat in rice cultivation. Discrimination of WR from cultivated rice is difficult since agro-morphology of WR and cultivated rice are overlapping. Molecular markers are useful and can be an informative tool for estimating genetic diversity and relationships in closely related WR eco-types. Self-Organizing Maps (SOM) is an interesting and promising classification tool employing an innovative and data-driven classification method based on unsupervised artificial neural networks. The present study focused on exploring the potential use of SOM to classify WR populations of different eco-climatic zones in Sri Lanka using agro-morphological and molecular data. Separate SOMs for each set of variables, agro-morphological and molecular data were developed. The best SOM was chosen based on the error performance. Findings of SOM analyses showed that certain morphological characters (seedling height, leaf blade width, leaf blade length, culm strength, panicle shattering, seed coat color and leaf angle) and certain molecular characters detected from SSR primers (RM 11, RM 21, RM 14, and RM 280) are important in separation of different WR eco-types satisfactorily. SOM clustering of cultivated, wild, and WR eco-types indicated specific patterns of grouping with respect to climatic conditions of the country. WR eco-types in dry zone and wet zone of the country are closely related to *Oryza nivara* and *O. rufipogon* respectively.

Keywords: Genetic diversity, *Oryza sativa* f. s*pontanea*, Self-Organizing Map, Sri Lanka, weedy rice

INTRODUCTION

Weedy rice (WR) (Oryza *sativa* f. *spontanea)* belongs to the Family Poaceae and is widely distributed in rice-planting areas all over the world, particularly in South and South-East Asia, South and North America, and Southern Europe (Mortimer et al. 2000; Chauhan and Johnson 2010; Chauhan 2012). Emergence of WR leads to high production costs, reduction of yield (Azmi et al. 2008) and lowered commodity value by staining the grains with undesirable pericarp color (Mortimer et al. 2000). Presently, WR has reached a considerable competitive level of infestation threatening rice cultivation sustainability, especially in Asian countries (Londo et al. 2007). WR was first reported in 1992 from Ampara District, Sri Lanka, and has spread into many areas of the country. WR occurs in all agro-ecological zones of the country with varying population densities (Abeysekara et al. 2010). Factors that contribute to the distribution of WR are: gene flow between wild and cultivated rice varieties, old rice varieties becoming feral, and crosses between cultivated rice and wild rice (Marambe et al. 2000; Karunarathna et al. 2014).

Microsatellite markers were coupled with morphological characteristics to explore genetic diversity and possible origins of weedy rice in Taizhou city, Jiangsu Province, China, With wild rice, hybrid rice and cultivated rice revealing low diversity amongst weedy rice populations and suggesting that weedy rice has originated from segregating populations of hybrid rice that hybridized naturally with cultivated rice (Zhang et al. 2008). Results of the molecular studies on the Sri Lankan weedy rice populations carried out by He et al. (2014) have shown that a high level of genetic diversity as estimated by 23 SSR loci, which is comparable with that estimated based on morphological characterization. The genetic features of weedy rice (*Oryza sativa* f. *spontanea*) have been studied based on morpho-physiology, random amplified polymorphic DNA (RAPD) (Suh et al. 1997), restriction fragment length polymorphism (RFLP) (Cho et al. 1995), amplified fragment length polymorphism (AFLP) (Federici et al. 2001) and simple sequence repeat (SSR) (Cao et al. 2006; Ma et al. 2008).

SSR markers have been identified as a powerful tool due to their abundance in eukaryotic genomes, co-dominance, and high polymorphisms (Gwag et al. 2010; Zhang et al. 2011) and thus, SSR markers have been used as allele-specific and co-dominant markers in population genetic and evolutionary studies of many plants (McKhann et al. 2004; Upadhyaya et al. 2006).

More recently, with the advancement of computer technology and algorithms, computerized keys with interactive systems have been used for plant identification which allows rapid identification and information retrieval. There have been several major approaches made to use computer algorithms in identification of biological specimens. These include computer-stored dichotomous keys; computer constructed keys, simultaneous characteristic-set methods, and automated pattern recognition (Dallwitz 1980). The Artificial Neural Network (ANN) has been developed to simulate the activity of the human brain (Simon 1999). Among the ANN learning algorithms, the Self-Organizing Map (SOM) is a learning algorithm that has been proposed by Kohonen (1982). A SOM algorithm also known as Kohonen Map or Self-Organizing Feature Map is an unsupervised neural network based on competitive learning (Kohonen 1998; Kohonen 2001). SOM is considered as an effective and innovative, data-driven classification method built on unsupervised Artificial Neural Networks (ANN) (Wang et al. 2014). SOM has been applied in varied disciplines such as agriculture and environment management due to prediction, estimation, classification, remote sensing image classification, etc. (Agarwal and Skupin 2008; Brosse et al. 2001). Further, SOM reduces the high-dimensional input data onto a low-dimensional space.

In general, SOM consists of two types of units *viz.*, an input layer and an output layer (input variables). The array of input units operates simply as a flow-through layer for the input vectors and has no further significance. SOM often consists of a two-dimensional network of neurons arranged in a square grid or lattice in the output layer. Each neuron is connected to its nearest neighbors on the grid. The neurons store a set of weights, each of which corresponds to one of the data inputs (Vesanto and Alhoniemi 2000). During these processes, weights are adjusted through iteration. The outcome weight vectors of the SOM nodes are allocated to have characteristic data patterns. An important feature of SOMs that distinguishes them from Vector Quantization techniques is that the neurons are organized on a regular grid. During training, both Best-Matching Neuron (BMU) and its topological neighbors are updated.

The comparison of results obtained from the SOMs with the results obtained from conventional multivariate statistical methods, such as PCA and correspondence analysis, revealed that SOM facilitates both visualizations of the sample units and finds underline structures in ecological communities (Lek and Gue gan 2000). Application of SOM in an environmental data set for predicting the species richness of aquatic insects in streams has been successfully achieved (Brosse et al. 2001). The SOM, the MLP, and a network based on the adoptive resonance theory (ART) used in animal science applications (Fernandez et al. 2006). Various algorithms (including k-means and fuzzy c-mean clustering techniques, SOM, and fuzzy adaptive resonance theory (ART) have been applied to the dive profiles of penguins and seals (Schreer et al. 1998). Unsupervised classification methods such as SOM (Li 2002) have been attempted to identify and classify crop weeds (Moshou et al. 2001; Hemming and Rath 2001). The long-term uses of SOM-assisted discrimination of weedy rice from cultivated rice can be potentially employed in monitoring edible rice cultivations, combating unwanted eco-types of rice, supporting weedy rice combustion with artificial intelligence tools used by drones, etc.

In agriculture, visualization of complex agro-morphological and even molecular data of different crop varieties in two-dimensional space is very important. In such situation, SOMs can be used as supplementary to conventional statistical analyses. However, there is scarcity in the studies that focus on complex patterns of agro-morphological and molecular characters across different rice varieties in different origins of ecology. The use of artificial intelligence systems in controlling weeds, including weedy rice in rice ecosystems, is important for widely distributed weeds. SOM provides a way to recognize the patterns reflected from the output data and the relationships between the input data. Thus, there are benefits of application of SOM to solve the problems of species identification *viz.* minimize the need for user interaction and improve the capability of the system to learn previously vaguely defined complex visual patterns (Walley et al. 2000).

The objective of this study was to explore the potential use of the SOM in classification of weedy rice populations that widely occur in different climatic zones of Sri Lanka using agro-morphological and molecular data.

MATERIALS AND METHODS

Agro-morphological characterization

The seeds of weedy rice eco-types, cultivated rice varieties, and wild rice varieties were collected from rice fields located in twelve (12) Districts (Ampara [12 WR eco-types], Anuradhapura [8 WR eco-types], Batticaloa [11 WR eco-types], Hambantota [11 WR eco-types], Kandy [10 WR eco-types], Kurunegala [15 WR eco-types], Matale [10 WR eco-types], Matara [24 WR eco-types], Mannar [10 WR eco-types], Polonnaruwa [10 WR eco-types], Puttalam [11 WR eco-types], and Vauniya [10 WR eco-types] in different eco-climatic zones (Dry, Wet and Intermediate zones) in Sri Lanka (Figure 1).

Collected seeds were subjected to dormancy, breaking treatments, and sown in plastic trays in a plant house at the Open University of Sri Lanka, Nawala, Sri Lanka. Five replicates each with a single plant were planted in plastic pots with paddy soils. Replicates were arranged in a Complete Randomized Design (CRD). Morphological characterization using thirty-six (36) characters of different rice varieties (Table 1) was made using the Standard Rice Characterization Catalogue (PGRC 1999).

Molecular characterization

Total genomic DNA was extracted from 7-day old seedlings of WR eco-types, cultivated rice varieties, and wild rice varieties using Ceygen Plant total DNA purification kit. Ten SSR primer pairs were used. The primer sequences and amplification conditions for primers were obtained from http://www. gramene.org/. A four-primer system (Schuelke 2000) was used, which included a universal M13 oligonucleotide (TGTAAAACGACGGCC AGT), labeled with one of four fluorescent dyes (6-FAM, NED, PET, or VIC) (Table 2).

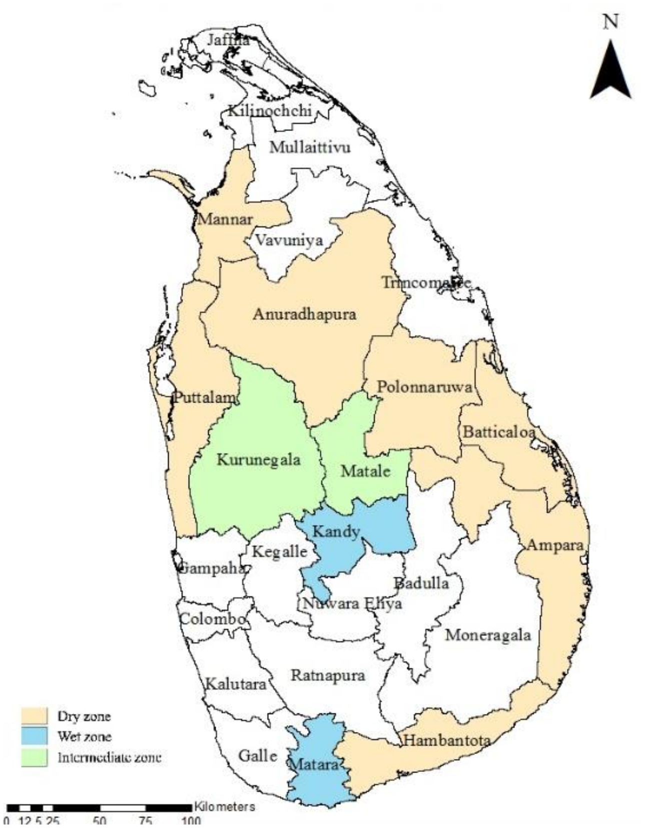

Figure 1. Geographical locations of the weedy rice populations sampled in Sri Lanka.

The fluorescent dyes allowed the products to be perplexed during electrophoresis; a special forward primer composed of a concentration of the M13 oligonucleotide; and the pigtail reverse primer for SSR PCR amplification. All amplification reactions were carried out in 30µl volumes of which contained 1 x PCR buffer, 1mM dNTPs, 2µM SSR primers, 2mM $MgCl_2$, 50ng of genomic DNA, and 0.5 Units of *Taq DNA polymerase*. The reaction conditions were: 95 ºC for 1min, followed by 30 cycles of 95ºC (30 sec), 55 ºC (1 min), and 72ºC (1 min), with 10 subsequent cycles of 95 ºC (30 sec), 53ºC (45 sec), and 72 ºC (1 min), and a final extension at 72ºC for 10 min. The SSR alleles were resolved on an ABI Prism 3100 DNA sequencer using GeneScan 4.1 software and sized precisely using GeneScan600LIZladder. Fragment analysis using capillary electrophoresis was performed using GENE MAPPER software and identified different peaks among weedy rice, cultivated rice varieties and wild rice varieties. Collected data were normalized (z-score) to have a zero mean and standard deviation of one (1).

Construction of Self-Organizing Map

A Self-organizing map (SOM) is a form of an ANN that can provide an objective way of classifying data through self-organizing networks of artificial neurons. It is a feed-forward ANN that uses an unsupervised training algorithm and can be trained to learn or find relationships between inputs or organize data to discover unknown patterns or structures. The SOM algorithm has been applied to a variety of real-world problems (Oja 2002). The main advantage of applying the algorithm comes from the easy visualization and interpretation of the map clusters. One of the main reasons (Kohonen 2002) for using a SOM for exploratory data analysis and data mining is that it is a numerical method and can treat numerical statistical data naturally and represent graded relationships. Other reasons for using the SOM algorithm include that it is a non-parametric method; no assumptions about the distribution of data need be made in advance, and it is a method that can detect unexpected pattern structures by learning without supervision. The SOM can be used deductively and is able to produce results even if the data set is incomplete, which is extremely important when dealing with biological material requiring identification. Another advantage of the SOM is that it can provide a probability for each species of an unidentified biological specimen belonging to a particular species. At the same time, the SOM can also be used to investigate the differences between clusters of species, and in the process, it is possible that some new features that are discern between the species might be revealed. The SOM can also be used to determine which features or characteristics are the most important or diagnostic ones to consider when discerning between given species.

The SOM training utilizes a competitive learning strategy during which a weighted vector associated with each neuron in a neural network is modified and is gradually developed to become sensitive to a set of patterns from a specific domain of the input space. The result of the training process is that different neurons specialize to represent different types of input patterns. This specialization is enforced by competition among the neurons. Neighboring neurons are allowed to learn, those neurons will also gradually specialize to represent similar patterns, and consequently, the representations on the output layer will become ordered (Kaski 1996). Usually, the neurons are arranged on a regular 1- or 2-dimensional lattice type of array with a hexagonal and oblong arrangement. This arrangement can represent the data clusters better than a rectangular arrangement and fits the data input distribution more easily (Deboeck and Kohonen 1998). The number and positions of neurons on the grid are defined and fixed, when the map is created and depends on the purpose for which the SOM will be used and the amount of input data. Sometimes the number of neurons used is determined by a heuristic formula such as $5\sqrt{N}$ or $\sqrt{N}$, where N is the number of training patterns (Vesanto 2000).

The SOM is defined in the training phase. During the training, assumptions must be made about several parameters of the map, such as learning parameters, map topology and map size. These features influence the final map; thus it is very important to choose these parameters carefully in order to reach the appropriate map (Kohonen 2001). Once tested with different choices, it is possible to use some measures to evaluate the quality of the map and select the optimal one to represent the experimental data. Several measures have been used to evaluate the quality of a SOM. A widely used measurement is the quantization error. This error measures the average distance between each data vector and its best matching unit (BMU). The quantization error (Eq) is calculated as shown in equation (1), where N is the number of data-vectors, X_i is the current vector patterns, m_c is the BMN of the corresponding X_i input vector.

Table 1. Agro-morphological characters used in characterization of WR eco-types cultivated rice varieties, and wild rice species in Sri Lanka (PGRC Rice Catalog 1999).

Character no.	Character	Abbr.	Description
1	Seedling height (cm)	Sdlh	Recorded at the five-leaf stage
2	Leaf-blade length (cm)	LBL	Measured from topmost leaf below the flag leaf on the main culm at late vegetative stage.
3	Leaf-blade width (mm)	LBW	Measured at the widest portion of the leaf blade
4	Leaf-blade pubescent	LBP	1. Glabrous, 2. Intermediate, 3. Pubescent
5	Leaf-blade color	LBC	1. Pale green, 2. Green, 3. Dark green, 4. Purple tips, 5. Purple margins, 6. Purple blotch, 7. Purple
6	Basal leaf sheath color	BLSC	1. Green, 2. Purple lines, 3. Light purple, 4. Purple
7	Leaf angle	LeafA	1. Erect, 2. Intermediate, 3. Horizontal, 4. Descending
8	Flag leaf angle	FleafA	1. Erect, 2. Intermediate, 3. Horizontal, 4. Descending
9	Ligule length (mm)	LiguleL	Measured at late vegetative stage
10	Ligule color	LiguleC	0. Absent 1. White, 2. Purple lines, 3. Purple
11	Collar color	CollorC	1. Pale green, 2. Green, 3. Purple
12	Auricle color	AuricalC	0. Abscent 1. Pale green, 2. Purple
13	Days of heading	DaysofH	No. Of days from effective seeding to, 50% heading
14	Culm length (cm)	CulmL	From ground level to the base of the panicle
15	Culm number	CulmNo	Total no. of grain bearing and non-bearing tillers
16	Culm angle	CulmA	1. Erect, 3. Intermediate, 5. Open, 7. Spreading, 9. Procumbent
17	Internode color After Full Heading	INCAF	1. Green, 2. Light gold, 3. Purple lines, 4. Purple
18	Culm strength	CulmS	1. Strong, 3. Moderately strong, 5. Intermediate, 7. Weak, 9. Very week
19	Panicle length (cm)	PanicleL	From the base to the tip of the panicle
20	Panicle type	PanicleT	1. Compact, 5. Intermediate, 9. Open
21	Secondary branching	SecB	0. Abscent 1. Light, 2. Heavy, 3. Clustering
22	Panicle exsertion	PanicleE	0. Well exsertion, 3. Moderately, 5. Justexterted, 7. Partlyexserted, 9. Enclosed
23	Awning after full heading	AwnAFH	0. Absent 1. Short and partly awned, 5. Short and fully awned, 7. Long and partly awned, 9. Long and fully awned
24	Apicus color	ApiculC	1. White, 2. Straw, 3. Brown, 4. Red, 5. Red apex, 6. Purple, 7. Purple apex
25	Lemma and palea color	LemmaPC	0. Straw, 2. Gold, 3. Brown spot on straw, 4. Brown, 5. Reddish to light purple, 6. Purple spots on straw, 7. Purple, 8. Black, 9. White
26	Lemma and palea pubescence	LPP	1. Glabrous, 2. Hairs on lemma keel, 3. Hairs on upper portion, 4. Short hairs, 5. Long hairs
27	Sterile lemmacolor	SLC	1. Straw, 2. Gold, 3. Red, 4. Purple
28	Sterile lemma length	SLL	1. Short, 3. Medium, 5. Long, 7. Extra-long, 9. Asymmetrical
29	100 grain weight	Gw100	A random sample of 100 well-developed grains dried 13% moisture content
30	Seed coat color	SeedCC	1. White, 2. Light brown, 3. Speckled brown, 4. Brown, 5. Red, 6. Variable purple, 7. Purple
31	Ligule shape at late vegetative stage	LiguleS	0. absent, 1. acute to acuminate, 2. 2-cleft, 3. truncate
32	Leaf senescence	Leaf S	1. Late and low senescence-, 2 or more leaves retain their green color at maturity, 5. Intermediate, 9. Early and fast senescence- leaves are dead when the grains have become fully ripened
33	Awn color at maturity	AwnC	1. Straw, 2. Gold, 3. Brown, 4. Red, 5. Purple, 6. Black
34	Panicle threshability	PanicleT	1. difficult, 5. intermediate, 9. easy
35	Panicle axis at maturity	PanicleA	1. Straight, 2. Droopy
36	Panicle shattering	PanicleS	1. Very low, 3. Low, 5. Moderate, 7. Moderately high, 9. High

Table 2. Four labeled primers used for the Capillary electrophoresis.

Oligo name	Oligo sequence (5'-3')	Color of the Label Primer
5'- FAM- M13 (-21)	5'(FAM) TGT AAA ACG ACG GCC AGT 3'	Blue
5'- NED- M13 (-21)	5'(NED) TGT AAA ACG ACG GCC AGT 3'	Yellow
5'- PET- M13 (-21)	5'(PET) TGT AAA ACG ACG GCC AGT 3'	Red
5'- VIC- M13 (-21)	5'(VIC) TGT AAA ACG ACG GCC AGT 3'	Green

$$Eq = 1/N \sum_{i=1}^{N} (Xi - mc) \quad (1)$$

This error evaluates the fitting of the neural map to the data. Thus, the optimal map is expected to yield the smallest average quantization error. The smaller the quantization error, the smaller the average of the distance from the vector data to the prototypes, and that means, that the data vectors are closer to their prototypes.

An alternative approach for measuring topology preservation is to use input samples to determine how continuous the mapping from the input space to the map grid is. One of the most extended indices for this purpose is the topographic error (Kiviluoto 1996). It is also one of the errors proposed by Kohonen (Kohonen 2001). This error measures the proportion of all data vectors for which first and second best-matching units (BMU) are not adjacent vectors. Therefore, the lower the topographic error (Et) is, the better the SOM preserves the topology.

$$Et = 1/N \sum_{i=1}^{N} U(Xi) \quad (2)$$

The topographic error is calculated as shown above, where the equation 2. U(**x**i) = 1, if the 1st BMN and 2nd BMN are not adjacent, otherwise U(**x**i) = 0. Separate SOMs for each set of variable *i. e*., morphological and molecular data were developed (SOMmor and SOMmol) (Uriarte and Martin 2005). The best SOM was chosen based on the error performance of quantization error (Eq) and topographic error (Et).

RESULTS AND DISCUSSION

There was a variation of agro-morphological characters (parametric only) across different WR eco-types in different eco-climatic zones in Sri Lanka. Analysis of variance of parametric variables used in construction of SOM for the morphological characters interactions with samples; climatic zones are shown in Table 3. According to Table 3, two (panicle length and 100-grain weight) out of eight parametric variables were not statistically significant ($p < 0.05$).

Similarly, χ^2 test of non-parametric variables shown in Table 4 indicated that two out of 28 variables such as leaf blade pubescence and culm number were not different across the rice populations. The summary of analysis of variance of molecular data of rice populations is shown in Table 5. According to the table, the markers RM11A1, RM21A1, RM21A2, RM44A1 RM44A2 and RM280 A2 were significantly varied across the rice populations (Table 5).

Different rice populations resulted from SOM analysis of morphological variables were grouped into labeled as Weedy rice populations in the dry zone - D1W, D2W, D3W, D4W and D5W; weedy rice populations in the intermediate zone - I1W and I2W; and weedy rice populations in the wet zone -WW1 and WW2. The group of cultivated rice resulting in the SOMmor, were also indicated by cultivated rice population in the dry zone – DC1, DC2, DC3; and cultivated rice population in the intermediate zone I1C and I2C; cultivated rice population in the wet zone – WC (*unpublished data*).

The analysis of variance performed on quantitative morphological data of SOM groups is shown in Table 6. Except, 100-grain weight, the rest of the variables were significantly between the weedy rice populations ($p < 0.05$). Similarly, all the qualitative characters of rice populations differed significantly between SOM groups of WR eco-type across the different climatic zones, and association between the climate zone and non-parametric variables included in the study (data not shown).

The resulting SOM grouping patterns of WR eco-type populations indicate that SOM can differentiate WR eco-type populations in different climatic zones of the country. The qualitative and quantitative variables were used to develop two SOM models, SOMmor and SOMmol for morphological and molecular data respectively. The error performance, Et and Eq of best SOMmor developed in the study were 0. 005 and 0. 580 respectively. The labels in SOM matrices (Figure 2) show the "position" of the data samples in the SOM plane. The component planes of agro-morphological characters (qualitative and quantitative) (Figures not shown) characters such as seedling height, leaf blade width, leaf blade length, culm strength, panicle shattering, seed coat color and leaf angle emerge as salient characters which distinguish weedy rice populations in the different climatic zones of the country.

The grouping pattern of WR eco-types in the label matrix (Figure 2) revealed a grouping trend associated with eco-climatic conditions of the country. Further, there was an association of WR eco-types such as AKW2, APW2, KIW2, ADW etc., which were closely related to *O. nivara,* a wild rice species commonly found in the dry zone of the country suggesting that *O. nivara* is potentially hybridized with weedy rice eco-types.

The results obtained in SOMmol (Eq = 0. 005 and Et = 0. 331) derived from the molecular data (Tamayo et al. 1999) of the rice populations were indicated in Figure 3. The comparison of component planes showed (Figures not given), indicated four primer pairs that reflect considerable variation among the populations of WR eco-types. Among the primer pairs used in the study, RM 11 A1, RM 21 A1, RM 21 A2, RM 14 A1 and RM 280A2 showed capability of identifying populations of WR eco-type along the climatic gradient of the country. The grouping pattern of rice populations produced by SOMmol is shown in Figure 3. (Label matrix). A total of three groups (dry, wet, and intermediate rice populations) were represented in the label matrix. WR eco-types in the dry zone were closely associated with *O. nivara* and wet zone WR eco-types are grouped with O. *rufipogon* reflecting their close relationships. Application of SOM with molecular data produced clear SOMmol groups than that of the SOMmor developed from the agro-morphological characters.

Table 3. The effect of parametric variables used in constructing SOMs.

Variable	Source	df	Mean square	F ratio	Sig. (p)
SDLH	Climate	2	1145. 604	24. 066	s
	Samples	2	2682. 572	56. 354	s
	Climate*Samples	3	158. 100	3. 321	s
LBL	Climate	2	248. 482	4. 100	s
	Samples	2	6552. 096	108. 112	s
	Climate*Samples	3	939. 210	15. 497	s
LBW	Climate	2	284. 242	28. 939	s
	Samples	2	13. 879	1. 413	ns
	Climate*Samples	3	40. 505	4. 124	s
PanilcL	Climate	2	1195. 631	52. 628	s
	Samples	2	961. 600	42. 327	s
	Climate*Samples	3	29. 202	1. 285	ns
GW100	Climate	2	. 324	. 352	ns
	Samples	2	3. 789	4. 119	s
	Climate*Samples	3	. 652	. 709	ns
LiguleL	Climate	2	125. 505	5. 232	s
	Samples	2	155. 722	6. 491	s
	Climate*Samples	3	301. 821	12. 582	s
DaysofH	Climate	2	2501. 815	53. 313	s
	Samples	2	1317. 238	28. 070	s
	Climate*Samples	3	1387. 490	29. 567	s
CulmL	Climate	2	2342. 816	47. 842	s
	Samples	2	1285. 035	26. 241	s
	Climate*Samples	3	539. 453	11. 016	s

Note: s: significant at $p \leq 0.05$, ns: not significant $p \geq 0.05$

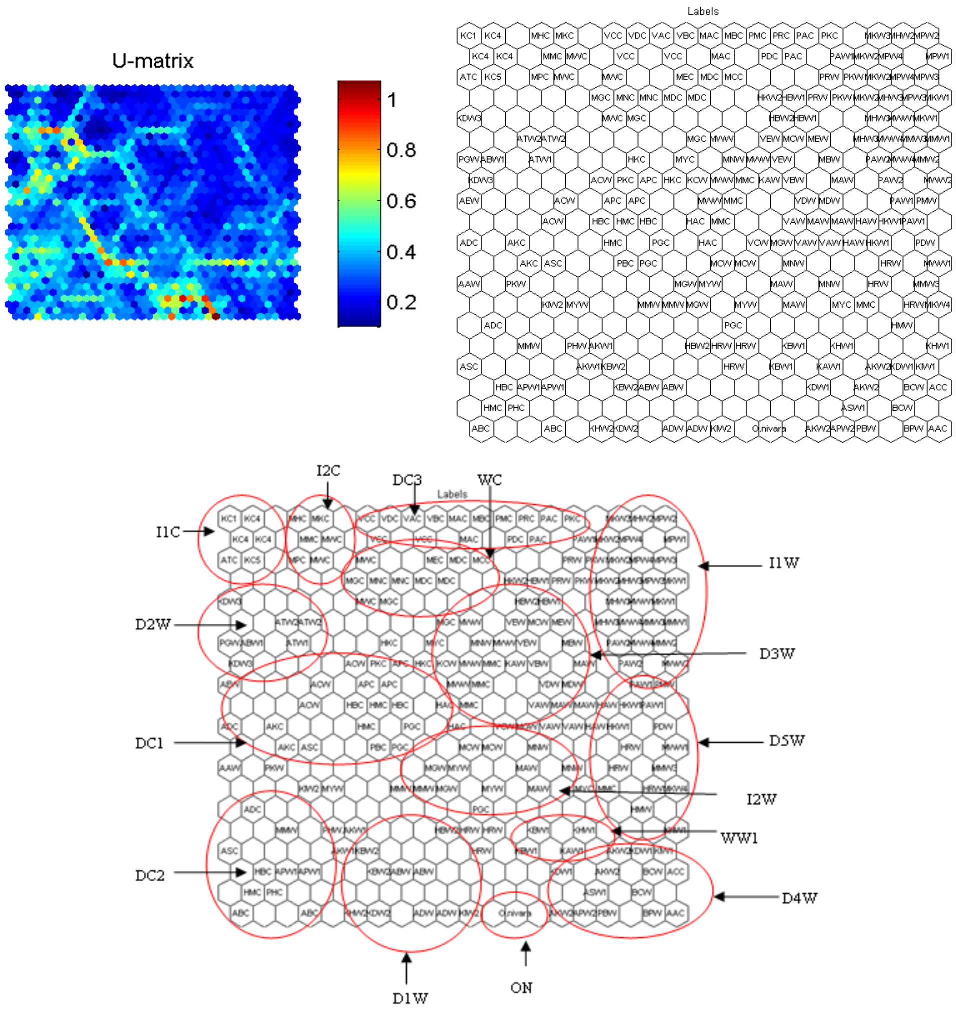

Figure 2. U matrix and the label matrix of the SOM developed from the agro-morphological characters.

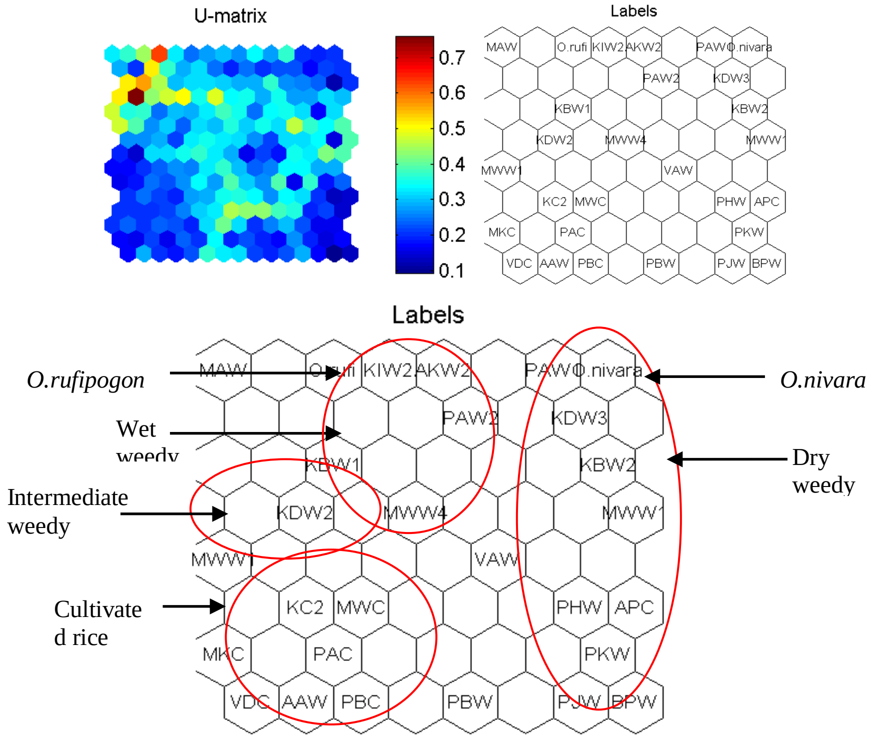

Figure 3. U matrix and label matrix of SOM developed for molecular data.

Table 4. The effect of climatic zone on qualitative morphological features of rice populations (CLIM).

Variable	χ^2 value	Df	Probability
BLSC*CLIM	43. 849	6	s
INCAF * CLIM	114. 251	6	s
LBC * CLIM	180. 398	10	s
LBP * CLIM	8. 175	4	ns
PanicleA * CLIM	22. 790	4	s
PanicleE * CLIM	65. 701	8	s
PanicleT * CLIM	50. 088	4	s
SecB * CLIM	26. 097	6	s
PanicleS * CLIM	119. 253	12	s
PanicleT * CLIM	143. 788	14	s
AwnAFH * CLIM	132. 786	8	s
AwnC * CLIM	124. 642	10	s
ApiculC * CLIM	52. 431	4	s
SeedCC * CLIM	17. 102	6	s
Leaf S * CLIM	191. 292	8	s
LemmaPC * CLIM	82. 196	14	s
LPP * CLIM	47. 384	4	s
SLC * CLIM	197. 225	4	s
SLL * CLIM	126. 854	4	s
LeafA * CLIM	32. 154	6	s
FLeafA * CLIM	232. 013	10	s
LiguleC * CLIM	36. 778	2	s
LiguleS * CLIM	32. 467	4	s
CollorC * CLIM	88. 948	4	s
AuricalC * CLIM	45. 764	2	s
CulmNo * CLIM	19. 738	16	ns
CulmA * CLIM	141. 045	10	s

Note: s: significant at $p \leq 0.05$, ns: not significant $p \geq 0.05$

The application of SOM analysis in grouping WR eco-types, with respect to eco-climatic zones of Sri Lanka, was carried out using agro-morphological and molecular data. The relationships among the agro-morphological characters (36 characters) and molecular data (derived from 10 primer pairs) were examined and generated hypotheses and tested. The SOM is supportive of other statistical analyses such a Principle Component Analyses, Cluster by complementing ability to examine the relationships between different types of variables in a visual presentation of the data(Mele and Crowley 2008). Visualization of the component planes derived from the SOM (SOMmor and SOMmol) indicates that there are patterns within the sets of variables. The SOMmor model developed indicated that seedling height, leaf blade width, leaf blade length, culm strength, panicle shattering, seed coat color, and leaf angle play an important role in grouping WR eco-type populations in Sri Lanka (Figure 3).

Meanwhile, the molecular banding patterns of primers such as RM 11 A1, RM 21 A1, RM 21 A2, RM 14 A1, and RM 280A2 revealed that they are important in grouping different populations of WR eco-types in Sri Lanka (Figures not given).

Table 5. The effect of variance of molecular data .

Marker	Sum of squares	df	Mean square	F ratio	Sig.
RM11 A1	16828. 725	2	8414. 363	3. 715	**s**
RM11 A2	593. 789	2	296. 895	0. 505	ns
RM21 A1	38527. 956	2	19263. 978	7. 727	**s**
RM21 A2	36618. 273	2	18309. 136	8. 884	**s**
RM14 A1	4731. 476	2	2365. 738	1. 054	ns
RM14 A2	7185. 571	2	3592. 786	2. 06	ns
RM44 A1	9823. 883	2	4911. 942	9. 322	**s**
RM44 A2	15850. 684	2	7925. 342	29. 516	**s**
RM84 A1	0. 265	2	0. 132	0. 002	ns
RM84 A2	30. 333	2	15. 167	0. 389	ns
RM167 A1	3576. 074	2	1788. 037	0. 926	ns
RM167 A2	3620. 583	2	1810. 292	0. 918	ns
RM205 A1	67. 681	2	33. 841	2. 417	ns
RM205 A2	46. 718	2	23. 359	1. 967	ns
RM211 A1	4943. 52	2	2471. 76	0. 457	ns
RM211 A2	5749. 015	2	2874. 507	0. 525	ns
RM280 A1	4666. 254	2	2333. 127	0. 535	ns
RM280 A2	4090. 331	2	2045. 165	0. 555	s
RM332 A1	148. 989	2	74. 495	0. 18	ns
RM332 A2	103. 283	2	51. 641	0. 009	ns

Note: s= significant at p ≤ 0. 05, and ns = not significant p ≥ 0. 05

Table 6. The effect of variance among the group of cultivated, wild rice species and weedy rice eco-types in Sri Lanka.

Variable	df	Mean square	F ratio	Sig.
Sdlh	15	933. 967	22. 146	s
LBL	15	1259. 932	27. 36	s
LBW	15	128. 407	15. 866	s
Gw100	15	1. 167	1. 119	ns
LiguleL	15	245. 899	11. 869	s
DaysofH	15	800. 506	20. 592	s
CulmL	15	1177. 008	40. 666	s

Note: s: significant at p ≤ 0. 05, ns: not significant p ≥ 0. 05)

The result of this preliminary study revealed that unsupervised artificial neural networks such as SOM are important tools in solving complicated biological problems associated with classification WR ecotypes with multivariate set of data. Further, results of the study led to hypothesis that the patterns reflected in the distribution of WR population are related to the varying climatic condition of the country. It appears that each WR eco-type represented each eco-climatic zone, especially closely associated with respective wild rice species. Further, WR eco-types of the dry and intermediate zones are closely related to *O. nivara*, commonly found in the country's dry zone. This observation suggests that the *O. nivara* possesses a higher potential of hybridization, with cultivated rice varieties and weedy rice eco-types in the dry and intermediate zones of Sri Lanka which is supported by the findings of Chen (Chen et al. 2004).

In conclusion, the finding of the study led to conclude that SOM is an important tool in tracing the patterns of variation of agro-morphological characters and molecular characters of WR eco-types with respect to the eco-climatic zones of the country. SOM can visualize the patterns of WR eco-type population distribution across different climatic conditions in a low-dimensional space. The characters such as seedling height, leaf blade width, leaf blade length, culm strength, panicle shattering, seed coat color, and leaf angle are the salient agro-morphological characters in identification of WR eco-type distributed in different eco-climatic zones of Sri Lanka. The primers such as RM 11, RM 21, RM 14, and RM 280 are of importance in delimitation of different WR populations in varying climatic conditions. The dimension reduction of input variables before presenting variables to SOM conserves the cost, labor, and computer processing time. The finding supports the idea capability of SOM in delimitation of WR populations through unsupervised artificial neural networks with limited multiple input variables. The findings of SOM analysis of the study's morphological data in the long run help develop intelligence systems to control weedy rice using areal targeting such as drones.

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