IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)
e-ISSN: 2319-2380, p-ISSN: 2319-2372. Volume 11, Issue 12 Ver. II (December 2018), PP 74-78
www.iosrjournals.org

Influenceof acetic acid, pH and buffers on *Striga hermonthica*seeds germination

Gafar, N.Y.[1]; Hassan, M.M.[1]; Rugheim A.M.E.[2]; Osman, A.G.[1]; Abdelgani, M.E.[1] and Babiker A.G.T.[1]

1 Environment, Natural Resources and Desertification Research Institute, the National Center for Research, Khartoum, Sudan.
2 Faculty of Agriculture, Omdurman Islamic University, Omdurman, Sudan.
Corresponding Author:Gafar, N.Y

Abstract: *This study was conducted to determine the effects of acetic acid, different buffers and their pH levels on Striga germination, early seedling growth and toxicity effect on Striga hermothica.Further the effects of pH on germination inducing activity and was determined. Results displayed that all acetic acid concentrations applied to Striga seed during conditioning significantly inhibited germination as compared to the control. Acetic acid at the lowest concentration (0.57µM) had an inhibitory effect as compared to the control. Furthermore, acetic acid at the highest concentration (5.7×10^{7} µM) completely inhibited germination (100 %) as compared to the control. However, un-diluted acetic acid (100%) caused complete inhibition of conditioned Striga seed when applied simultaneously with GR24. Striga germination decreased with increasing acetic acid concentration, irrespective of GR24 concentration. pH had consistent effects on germination inducing activity of the parasite. Striga seeds conditioned in sodium acetate (pH 9.3) and treated with GR24 reduced germination significantly by 79 % as compared to the water control. Acetic acid (pH 2.2) caused complete inhibition of germination. Moreover, all pH levels of acetate buffer inhibited germination by 83-100% in response to GR24 as compared to the control. Moreover, all buffers with different pH levels reduced germination significantly in response to GR24. Generally, the depressive effects of buffers were decreased with increasing GR24 concentration.*

Keywords*: Acetic acid, Buffer, Germination, pH, Striga*

Date of Submission: 16-12-2018 Date of acceptance: 31-12-2018

I. Introduction

Vinegar, with acetic acid (CH3COOH) as its main component, is potential as a natural herbicide added that acetic acid does not persist in the environment, but perishable with producing water as a byproduct [1].The control of weeds using herbicides could be a good method as it will cut the costs, time, labor, and control the weeds. The ideal herbicides are expected to have not toxic to the plants, effective to control the weeds, inexpensive and leave no negatively impact to the environment. Organic herbicides are biodegradable and act as carbon source for soil microorganisms.Acetic acid in the soil provides a source of carbon for thedecomposition process in producing carbon dioxide [2]. According to [3]reported that the acetic acid was absorbed into the plant and translocate toother parts plant of the inflict damage, therefore, it was considered to be a contact and as post-emergence herbicide as glyphosate. Further [4] added that there is a possibility reason that application of acetic acid may cause degradation of themembrane protein.

The root parasitic weed *Striga hermonthica*, a major constraint to sorghum and pearl millet production in sub-Saharan Africa [5]. *Striga* drain essential organic and inorganic resources from their hosts leading to poor crop development and low yield. Although improved cultural practices, herbicide use, and growing resistant varieties have been used to control the parasite [6], the *Striga* problem still remains unsolved to date. The parasite, therefore, is ranked as the leading biotic constraint to cereal production in Africa [7] where it has caused considerable loss in crop yield quantity and quality.The need for simple cost-effective, environmentally benign management practices which enhance seed bank demise and improve soil fertility and crop yield is thus imperative. This work was carried out to investigatesi) the effects of acetic acid at different concentration on *Striga* germination ii) effects of different buffers and their pH levels on *Striga* seeds germination**.**

II. Materials And Methods

Series of experiments were conducted to study the effects of acetic acid and buffers on *Striga* seeds germination in response to GR24**.**All laboratory experiments were conducted at the Department of Alternatives to Pesticides and Biological Control, Environment and Natural Resources Research Institute (ENRRI), National Center for Research (NCR), Khartoum, Sudan.

Striga hermonthica seedscollected from a sorghum field in Sinnar, Sudan in 2008 were used in this study.

GR24 stock solution

The strigolactone analogue GR24 was provided by professor Zwanenberg, B. the University of Nimijhen, the Netherlands. A stock solution (10 ppm) of GR24 was prepared by dissolving 1 mg in 1 ml of acetone and completing to volume (100 ml) with sterile distilled water. The solution was kept refrigerated at 4ºC for further use.

Acetic acid glacial

Acetic acid glacial (CH_3CO_2H) was purchased from the local market with a concentration of 0.995 g/ml.

Laboratory experiments

Striga seeds were surface sterilized according to the method described by[8]. Seeds were surface disinfected by socking in 70% ethanol for 2min. with continuous agitation and rinsed three times with sterile distilled water. Subsequently, the seeds were immersed in 1 % sodium hypochlorite for 2min. and rinsed three times with sterile distilled water. The NaO_2Cl solution was obtained by dilution of commercial (5%) sodium hypochlorite (Bleach) solution. Subsequently, the seeds were thoroughly washed with sterilized distilled water. The seeds were plotted dry on Whatman filter paper (No.1) under a laminar flow hood, then were kept in sterilized vial for further studies.

Striga seed preconditioning and germination

Striga seeds were conditioned as described by [9]. Briefly, glass fiber filter papers (GF/C) discs (8mm diameter) were cut, wetted thoroughly with water and placed in an oven at 100ºC for 1h to be sterilized. The discs, placed in 9 cm Petri dishes lined with glass fiber filter papers (GF/C), were moistened with 5 ml distilled water. About 25 - 50 surface disinfected *S. hermonthica* seeds were sprinkled on each of the glass fiber discs in each Petri dish. Aluminium foil was used to enclose the dishes to exclude light. The seeds were then preconditioned for 10 days in the dark at 30°C before use in relevant experiments.

The disc containing *Striga* seeds were dapped on normal filter paper (No.1) to remove excess water and then transferred to sterile Petri dishes. Each disc was treated with 20µl of GR24 at 0.01 and/or 0.1ppm. Then the seeds were re-incubated and examined for germination 24h later using a stereomicroscope. A *Striga* seed was considered to have germinated if it showed a protruded radicle through the seed coat

Effects of acetic acid and buffers on S. hermonthica seeds germination in response to GR24

Two experiments were conducted to study the effects of acetic acid on *Striga* seeds germination in response to GR24. In the first and second experiments, eight concentrations (0.00001, 0.0001, 0.001, 0.01, 0.1, 1, 10 and 100%) of acetic acid were evaluated on *Striga* seeds germination applied during and after conditioning, as described above, then each disc was treated with 20µl of GR24. The seeds were re-incubated at 30ºC, and then examined for germination 24h. later using a binocular stereomicroscope.

Effects of different buffers on S. hermonthica seeds germination in response to GR24

Different buffers and pH levels were evaluated on *Striga* seed germination in response to GR24. *Striga* seeds conditioned in water were treated with different buffers in different levels of pH viz, acetate buffer (CH_3CO_2H, CH_3CO_2Na)with pH levels 3, 3.5, 4, 4.5, 5, 5.5 and 6, phosphate– borax buffer (KH_2PO_4, $Na_2B_4O_7$) with pH levels 8, 8.5, 9, ammonium hydroxide–ammonium chloride buffer (NH_4OH, NH_4CL) with pH levels 8, 8.5 and 9 and citrate–phosphate buffer ($C_6H_8O_7$, Na_2HPO_4) with pH levels 3, 4, 5, 6, 7 and 8.All salts and acids used in buffers preparation were included as controls. Only acetate buffer with different pH levels were investigated during conditioning *Striga* seeds as describe above. The disc containing *Striga* seeds were treated with GR24 at 0.1 ppm or distilled water. The seeds were re-incubated and examined for germination as described above.

Statistical analysis

In all laboratory experiments, treatments were arranged in a randomized complete design with 4 replicates. Data on percentage germination and haustorial initiation were calculated for each disc, transformed to arcsine and subjected to analysis of variance (ANOVA). Meanswere tested for significance by the Duncan Multiple Range Test at $P \leq 0.05$.

III. Results And Discussion

Effects of acetic acid applied during condition and at terminate on S. hermonthica germination in response to GR24

Various concentrations of acetic acid treatments were evaluated on *Striga* seeds germination. GR24 applied to seeds conditioned in water induced the highest germination (68 - 71%) (Table 1). Results displayed that all acetic acid concentrations applied to *Striga* seed during conditioning significantly ($P \leq 0.05$) inhibited germination as compared to the control. Acetic acid at the lowest concentration [(0.00001 %) = 0.57µM] had an inhibitory effects as compared to the control. It significantly ($P \leq 0.05$) reduced germination by 29%, irrespective of GR24 concentration. Furthermore, acetic acid at the highest concentration [(stock 100 %) = 5.7×10^7µM] completely inhibited germination (100%) as compared to the control.

Striga seeds conditioned in water and treated with GR24 displayed higher germination (Table 2). Un-diluted Acetic acid (100%) caused complete inhibition of conditioned*Striga* seed when applied simultaneously with GR24. However, seeds treated with acetic acid at the lowest concentration (0.00001%) exhibited germination comparable as compared to the water control. Generally, *Striga* germination decreased with increasing acetic acid concentration, irrespective of GR24 concentration.[10]reported that 10 % acetic acid applied as pre-planting in wheat could control broadleaf weed.

Effects of buffers and pH on S. hermonthica seeds germination in response to GR24 (during and after conditioning)

GR24 (0.01 and 0.1 ppm) applied to seed conditioned in water induced high germination (72 - 85%) (Table 3). However, germination of *Striga* seeds conditioned in sodium acetate (pH 9.3) and treated with GR24 was significantly ($P \leq 0.05$) reduced by 79% as compared to the water control. Results displayed that acetic acid (pH 2.2) caused complete inhibition of germination. Moreover, all pH levels of acetate buffer except pH 6 completely inhibited germination in response to GR24 as compared to the control. At pH 6 of acetate buffer significantly ($P \leq 0.05$) decreased germination by 83%, irrespective of GR24 concentration as compared to the water control. Results showed that *Striga* seeds conditioned in water and similarly treated with GR24 sustained the highest germination (74 and 85%) (Table 4). Germination of *Striga* seeds conditioned in water for 10 days then subjected to sodium acetate (9.3 pH) or acetic acid (2.2 pH) for 3 days and treated with GR24, was reduced by 16 and 100%, respectively as compared to the water control. Germination of seeds conditioned at different pH levels of acetate buffer (3 to 4.5) was completely inhibited in response to GR24 as compared to water control. The effects of the rest pH levels (5 to 6) decreased with decreasing levels. Results displayed that all buffers with different pH levels significantly ($P \leq 0.05$) reduced germination in response to GR24 as compared to the control (Table 5). Generally, the depressive effects of buffers were decreased with increasing GR24 concentration. Germination of seeds conditioned in potassium dihydrogen phosphate (pH 4.1) and borax (pH 8) displayed significantly ($P \leq 0.05$) reduced germination by 25 and 100%, respectively, irrespective of GR24 concentration as compared to the water control. Furthermore, the decrease in activity of root exudates at high and low pH when taken in conjunction with the lactonic nature of *Striga* natural germination stimulants and their ubiquitous distribution in plants [11] are in line with instability of strigolactones in alkaline and acidic media as previously reported by [9 and 12]. A more direct effect on the germination and attachment/penetration of *S. hermonthica* to sorghum roots through alteration of the quantity or quality of sorghum root exudates is to be considered as well.However, germination of conditioned seeds treated with phosphate-borax buffer pH levels (8, 8.5 and 9) was completely inhibited. Moreover, germination of seeds conditioned in ammonium hydroxide solution (pH 11.1) or ammonium chloride (pH 4.5) was completely inhibited in response to GR24 as compared to water control. Furthermore, different pH levels of ammonium hydroxide - ammonium chloride buffer (8, 8.5 and 9) completely inhibited germination in response to GR24 as compared to water control. Results displayed that disodium hydrogen phosphate (pH 7.4) or citric acid (pH 2) significantly ($P \leq 0.05$) reduced germination by 52 and 100%, respectively in response to GR24 as compared to the water control. Different pH levels (3 and 4) of citrate - phosphate buffer completely inhibited germination in response to GR24 as compared to the water control. Moreover, the reduction effects on *Striga* seed germination decrease with decreased acidity. However, at neutral pH (7) and alkaline (pH 8), germination was reduced by 70 and 74%, respectively as compared to the control.[8]reported that ammonium nitrate and potassium phosphate applied to *Striga* seeds in different concentrations significantly ($P \leq 0.05$) reduced and delayed germination as compared to the control.[13]reported that pH lower than 4 or higher than 10 affected germination of lettuce and poppy seeds. In the present study a negative relationship was observed between different buffers and pH on germination of *Striga* seeds. The inhibitory effect of buffer pH on seed germination could be due to direct toxic effect on seeds and/or to the osmotic effect that prevents the seeds from imbibition[14]. [15]reported that pH is one of factors that affect the stability of enzymes. Hence, it can be concluded that the effect of buffer pH on the germination of *Striga* seed may be due to some biochemical changes occurring within the seeds and/or to direct effect on one or more enzyme(s) function.

Limited study shown that acetic acid may have potential as a natural herbicide.The control of parasitic weeds using acetic acid or acetic acid bacteria could be a good method as it will cut the costs and timeto control *Striga*. Acetic acid is an ideal herbicide easily decomposable by microorganisms and shows potential of bioaccumulation. However, more subtle interactions involving penetration and accumulation of the acetic acid in the seeds or differential activity of certain enzymes in the seeds cannot be ruled out. Further research is needed in order to evaluate the acidpersistence and stability in soil and various parts of the plant.

References

[1]. Diaz P. 2002. Vinegar of Organic Weed Killers. http:// www.epa.gov/pesticide/food/organics.htm. Accessed 27 February 2011.

[2]. Evans, G. J. Bellinder, R. R. and Hahn, R. R. 2011. Integration of Vinegar for In-Row Weed Control in Transplanted Bell Pepper and Broccoli. Weed Technology: July-September. 25(3): 459-465.

[3]. Webber, C.L. and Shrefler J.W. 2006. Vinegar as a burn-down herbicide: Acetic acid concentrations, application volumes, and adjuvants. 2005 Vegetable Weed Control Studies, Oklahoma State University, Division of Agricultural Sciences and Natural Resources, Department of Horticulture and Landscape Architecture. Stillwater, OK. MP-162, pp. 29-30.

[4]. Spancer D.F. and G.G. Ksander. 1997. Dilute Acetic Acid Exposure Enhances Electrolyte Leakage by Hydrillaverticillata and Potamogetonpectinatus Tuber. Journal Aqual Plant Manage. 35: 25-30.

[5]. Sauerborn, J., D. Müller-Stöver, and J. Hershenhorn. 2007. The role of biological control in managing parasitic weeds. *Crop Protection* 26(3), 246–254.

[6]. Scholes, J. D. and M. C. Press. 2008. *Striga* infestation of cereal crops - an unsolved problem in resource limited agriculture. *Current Opinion in Plant Biology* 11(2), 180–186.

[7]. Ejeta, G. and L. G. Butler. 1993. Host-parasite interactions throughout the *Striga* life cycle, and their contributions to *Striga* resistance. *African Crop Science Journal* 1(2), 75–80.

[8]. Hassan, M. M., Sugmuto, Y., Babiker, A. G. T., Yamauchi, Y., Osman, M. G. E., Yagoub, S. O. 2010. Effect of NaCl on Orobanche spp and Striga hermonthica seeds germination during and after conditioning. Bioscience Research, 7(1): 26-31**.**

[9]. Babiker,A. G. T. and A. M. Hamdoun 1982 Factors affecting activity of GR7 in stimulating germination of *Striga hermonthica* (Del.) Benth. *Weed Res.* **22**, 111- 115

[10]. Johnson E N., Wolf T M. and B. C. Caldwell. 2004. Vinegar for Pre-Seed and Post-Emergence Control of Broadleaf Weeds in Spring Wheat (*Triticumaestivum* L.). Proc. 2003 Nat. Meet.Canadian Weed Sci. Soc. 57th Annual Meeting. Halifax, Nova Scotia, Canada. 57: 87.

[11]. Yoneyama, K.; Ruyter-Spira, C.; Bouwmeester, H. 2013. Induction of germination. In: Joel, D. M.; Gressel, J.; Musselman, L. J. (eds) *Parasitic Orobanchaceae Parasitic Mechanisms and Control Strategies*pp 164-194.

[12]. Johnson, A. W.; Roseberry, G. and Parker, C. 1976. A novel approach to *Striga* and *Orobanche* control using synthetic germination stimulants. Weed Research 16:223-227.

[13]. Powers M. K. 2004. The Effect of pH on Seed Germination.California state science fair project summary. Project number J 1422.

[14]. Tobe, K.; Zhang, L. and Omasa, K. 1999. Effect of NaCl on seed germination of five non-halophytic species from a Chinese desert environment.*Seed Science and Technology***27:** 851 - 863.

[15]. Kumar, K. S.; Varma, G. M.; Kiran, A. V.; Mahesh, E., Kanth, D. L.; Sekhar, C. S. andParvathi, A. L. 2012. Industrially Produced Enzymes and their Applications-An Overview.*International Journal of Pharmaceutical and Chemical Sciences* **1:** 1069 - 1072.

Table 1 Effects of acetic acid, applied during conditioning on *S. hermonthica* seeds germination in response to GR24

GR24 (ppm.)	Germination (%)									
	Water	Acetic acid concentration %								mean
		0.00001 (0.57 µM)	0.0001 (57 µM)	0.001 (5.7×10^{2} µM	0.01 (5.7×10^{3} µM	0.1 (5.7×10^{4} µM	1 (5.7×10^{5} µM	10 (5.7×10^{6} µM	100 (5.7×10^{7} µM	
0.01	(56)	(36)	(30)	(29)	(26)	(0)	(3)	(0)	(0)	(20)
	68	35	28	26	20	0	1	0	0	20
0.1	(58)	(45)	(35)	(31)	(26)	(7)	(0)	(0)	(0)	(22)
	71	49	33	27	20	3	0	0	0	23
Mean	$(57)^{e}$	$(40)^{d}$	$(33)^{bc}$	$(30)^{bc}$	$(26)^{b}$	$(3)^{a}$	$(1)^{a}$	$(0)^{a}$	$(0)^{a}$	
	69	42	30	27	20	1	0	0	0	

Values between parenthesis ( ) indicate arcsine transformed data.

Means within a row followed by the same letter (s) are not significantly different according to Duncan Multiple Range Test at P $\leq$ 0.05.

S.E. for acetic acid (±3.1) **S.E.** for concentration (±1.4) **S.E.** for interaction (±4.4)

Table 2 Effects of acetic acid, applied at termination on *S. hermonthica* seeds germination in response to GR24

GR24 (ppm.)	Germination (%)									
	Water	Acetic acid concentration %								Mean
		0.00001 (0.57 µM)	0.0001 (57 µM)	0.001 (5.7×10^{2} µM	0.01 (5.7×10^{3} µM	0.1 (5.7×10^{4} µM	1 (5.7×10^{5} µM	10 (5.7×10^{6} µM	100 (5.7×10^{7} µM	
0.01	(49)	(48)	(37)	(36)	(25)	(19)	(0)	(0)	(0)	(24)
	57	56	37	35	18	10	0	0	0	24
0.1	(53)	(49)	(41)	(39)	(31)	(29)	(0)	(0)	(0)	(27)
	64	57	43	40	26	23	0	0	0	28
Mean	$(51)^{e}$	$(49)^{e}$	$(39)^{d}$	$(38)^{d}$	$(28)^{c}$	$(24)^{b}$	$(0)^{a}$	$(0)^{a}$	$(0)^{a}$	
	61	56	40	37	22	17	0	0	0	

Values between parenthesis ( ) indicate arcsine transformed data.

Means within a row followed by the same letter (s) are not significantly different according to Duncan Multiple Range Test at P $\leq$ 0.05.

S.E. for acetic acid (±1.9) **S.E.** for concentration (±1) **S.E.** for interaction (±2.7)

Table 3 Effects of acetate buffer and pH on *S. hermonthica* seeds germination in response to GR24 (during conditioning)

GR24 (ppm.)	Germination (%)										
	Water	A[1] 2.2	Na [2] 9.3	Acetate buffer pH levels							Mean
				3.5	3.5	4	4.5	5	5.5	6	
0.01	(59)	(0)	(12)	(0)	(0)	(0)	(0)	(0)	(0)	(8)	(8)
	72	0	4	0	0	0	0	0	0	4	8
0.1	(68)	(0)	(15)	(0)	(0)	(0)	(0)	(0)	(0)	(14)	(10)
	85	0	7	0	0	0	0	0	0	6	10
Mean	(63)^{c}	(0)^{a}	(13)^{b}	(0)^{a}	(0)^{a}	(0)^{a}	(0)^{a}	(0)^{a}	(0)^{a}	(11)^{b}	
	79	0	6	0	0	0	0	0	0	5	

[1]acetic acid, [2] sodium acetate. Values between parenthesis ( ) indicate arcsine transformed data.
Means within a row followed by the same letter (s) are not significantly different according to Duncan Multiple Range Test at P $\leq$ 0.05.
S.E for acetate buffer (±1.5) **S.E** for concentration (±0.6) **S.E.** for interaction (±2.1)

Table 4 Effects of acetate buffer and pH on *S. hermonthica* seeds germination in response to GR24 (after conditioning)

GR24 (ppm.)	Germination (%)										
	Water	A[1] 2.2	Na[2] 9.3	Acetate buffer pH levels							mean
				3	3.5	4	4.5	5	5.5	6	
0.01	(60)	(0)	(51)	(0)	(0)	(0)	(0)	(9)	(33)	(49)	(20)
	74	0	60	0	0	0	0	4	30	55	22
0.1	(68)	(0)	(57)	(0)	(0)	(0)	(0)	(24)	(35)	(57)	(24)
	85	0	70	0	0	0	0	18	33	67	27
mean	(64)^{e}	(0)^{a}	(54)^{d}	(0)^{a}	(0)^{a}	(0)^{a}	(0)^{a}	(17)^{b}	(34)^{c}	(53)^{d}	
	79	0	65	0	0	0	0	11	31	61	

[1]acetic acid, [2] sodium acetate Values between parenthesis ( ) indicate arcsine transformed data.
Means within a row followed by the same letter (s) are not significantly different according to Duncan Multiple Range Test at P $\leq$ 0.05.
S.E. for acetate buffer (±3.5) **S.E.** for concentration (±1.6) **S.E.** for interaction (±4.9)

Table 5 Effects of buffers and pH on *S. hermonthica* seeds germination in response to GR24 (after conditioning)

GR24 (ppm)	Germination (%)																			
	Buffers components with pH levels																			
		Phosphate–Borax						NH$_4$OH-NH$_4$CL						Citrate –Phosphate						
	W[1]	KH_2PO_4 4.1	$Na_2B_4O_7$ 8	8	8.5	9	NH_4OH 11.1	NH_4CL 4.5	8	8.5	9	Na_2HPO_4 7.4	citric acid 2	3	4	5	6	7	8	Mean
0.01	(50)	(36)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(20)	(0)	(0)	(0)	(3)	(6)	(7)	(6)	(7)
	58	35	0	0	0	0	0	0	0	0	0	16	0	0	0	1	2	3	2	6
0.1	(48)	(38)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(26)	(0)	(0)	(0)	(6)	(12)	(22)	(19)	(9)
	55	37	0	0	0	0	0	0	0	0	0	19	0	0	0	2	7	16	15	8
mean	(49)^{g}	(37)^{f}	(0)^{a}	(0)^{a}	(0)^{a}	(0)^{a}	(0)^{a}	(0)^{a}	(0)^{a}	(0)^{a}	(0)^{a}	(23)^{e}	(0)^{a}	(0)^{a}	(0)^{a}	(4)^{ab}	(9)^{bc}	(15)^{d}	(13)^{cd}	
	57	36	0	0	0	0	0	0	0	0	0	18	0	0	0	2	5	9	9	

[1]Water conditioning medium. Values between parenthesis ( ) indicate arcsine transformed data.
Means within a row followed by the same letter (s) are not significantly different according to Duncan Multiple Range Test at P $\leq$ 0.05.
S.E. for buffers (±2.4) **S.E.** for concentration (±0.8) **S.E.** for interaction (±3.5)

Gafar, N.Y." Influence of acetic acid, pH and buffers on Striga hermonthica seeds germination. "IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS) 11.12 (2018): PP 74-78.