

Growth, Carbon Assimilation and Quality of Kesum (*Persicaria Minor*) as Exposed to Zinc Oxide Nanoparticles

ABSTRACT

Aims: This study was conducted to investigate the effect of zinc oxide nanoparticles towards the *Persicaria minor* that can be used as a guidance for further toxicity investigation of ZnO-NPs.

Study design: A Completely Randomized Block Design (RCBD) was used with three replication. Each unit was consisted with eight plants and the total of 96 plants were used in this study.

Place and Duration of Study: This study was conducted in plot 1, Vegetables Field plot for Teaching and Research, Taman Pertanian Universiti, Universiti Putra Malaysia (UPM) Selangor, Malaysia, from May 2018 until August 2018.

Methodology: *Persicaria minor* were exposed to four different concentration of zinc oxide nanoparticles (ZnO-NPs) which were (50,100 and 150 mg/L) and 0 mg/L as a control. The ZnO-NPs was dissolved in distilled water before being applied to plants. 40 mL of ZnO-NPs solution was applied to each plant. The growth, carbon assimilation and also secondary metabolites were measured in this experiment.

Results: The results showed that the treatment of zinc oxide nanoparticles enhanced growth of the *Persicaria minor* as the plant treated with zinc oxide nanoparticles have higher plant height and total biomass when compared to control treatment. However, the analysis revealed that the treatment of zinc oxide nanoparticles highly and significantly influenced the carbon assimilation and quality of this plant as the treated plants showed reduction in chlorophyll content, photosynthesis rate, stomatal conductance and transpiration rate but increased in production of secondary metabolites. The increased in production of plant secondary metabolites may be attributed by the plant protection mechanism due to metabolic stress caused by high concentration of zinc oxide nanoparticles.

Conclusion: This research will progressively help in contributing some reliable and valid data on the effect of zinc oxide nanoparticles (ZnO-NPs), towards the *Persicaria minor* that can be used as guidance for further experimental investigation regarding this field.

12
13
14
15
16

Keywords: Persicaria minor, zinc- oxide nanoparticles, growth, carbon assimilation, secondary metabolites, toxicity

1. INTRODUCTION

17
18
19
20
21
22

According to National Health Portal [1], the World Health Organization (WHO) estimated that 80 percent of the world's population consuming herbal medicine for their health care and there are about 21000 plants species have potential to be utilized as medicinal plants. *Persicaria minor* is one of the plants that gained great attention in this field of study. According to Christopher et al. [2], *Persicaria minor* have gained great attention in scientific study due to its high content of antioxidant. This plant possesses variety of pharmacological

23 properties such as antioxidant activity, antiulcer activity, anti-inflammatory activity,
24 antimicrobial activity, anticancer activity and can enhance the digestive properties and
25 cytotoxic activity [2]. According to Vikram et al. [3], *Polygonum minus* has been used
26 traditionally in herbal medicine to treat digestive disorder, remove dandruff and the essential
27 oil that extracted from *Persicaria minor* leaves is used as aroma therapy and also in perfume
28 industry.

29
30 Recently, nanoparticles (NPs) are widely studied because its beneficial properties in
31 agriculture and allied sector [4]. According to them, zinc oxide nanoparticles (ZnO-NPs) is
32 one of NPs that used widely as it been utilized in variety of industrial sector including
33 medication, cosmetic materials, opposed microorganisms and textile industries. As it has
34 been commercially used, the toxicity effect of these ZnO-NPs to the environment and also
35 soil ecosystem are mainly concerned [5]. Sabir et al. [6] stated that ZnO-NPs possess
36 significant characteristics which have antimicrobial, optical and physical properties therefore
37 it have great potential to enhance agriculture. The presence of ZnO-NPs may enhance the
38 antioxidant mechanism that helps to stabilize the plants and improve the photosynthetic
39 efficiency [7]. However, the effect is depend on the concentration of ZnO-NPs and it is varies
40 from plant to plant [8].

41
42 Secondary metabolites are the natural compound that produced by the plants and some of
43 the compound are utilized as medicines, flavoring and drugs. According to Biology
44 Reference [9], the simple classification of plants' secondary metabolites includes three main
45 groups which are terpenes, phenolics and nitrogen- containing compounds. Secondary
46 metabolites does not involve in the plant growth and development but required for plant to
47 survive in the environment because they give negatives impact on other organisms such as
48 pathogen and herbivores that can harm the plants [9]. Secondary metabolites possess
49 significant biological properties and also medicinal importance that can improve
50 pharmaceuticals field [10].

51
52 There is no previous study being conducted on the effect of ZnO-NPs on physical and
53 biochemical response of *Persicaria minor* and also only few research being carried out to
54 discover about the effect of ZnO-NPs on carbon assimilation and production of secondary
55 metabolite of this plant, so this present study will reveal about the effect of ZnO-NPs towards
56 the *Persicaria minor* that can be the guidance to improve the field of agriculture in planting
57 this important medicinal plant and also other herb that being importantly used recently in
58 medicinal field and also be used as guidance for further toxicity investigation of ZnO-NPs.
59 Hence, the objectives of this study were to study the growth, carbon assimilation and quality
60 of *Persicaria minor* as affected by zinc oxide nanoparticles, to determine the optimum
61 concentration dose of zinc oxide nanoparticles that can enhance the optimum growth and
62 secondary metabolites of *Persicaria minor* and to recognize the relationship between
63 secondary metabolites and growth of *Persicaria minor* as exposed by zinc oxide
64 nanoparticles application.
65

66

67 **2. MATERIAL AND METHODS**

68

69

70 **2.1 Experimental site**

71

72 This study was conducted in plot 1, Vegetables Field plot for Teaching and Research,
73 Taman Pertanian Universiti, Universiti Putra Malaysia (UPM) Selangor. The research site
74 was set up with net shading and black plastic to reduce the absorption of water by sunlight
75 since *Persicaria minor* require high amount of water and also to reduce the competition with
76 grasses and other plants. This experiment was conducted from the month of May 2018 until
77 August 2018.

78

79 **2.2 Planting material**

80

81 *Persicaria minor* was obtained from Jabatan Pertanian, Serdang. The first internodes at the
82 bottom of the shoots with five number of leaves was cut in 5cm in height for the shoot stem
83 cutting preparation. The shoot stem cuttings were immersed in the tap water overnight to
84 increase their turgidity which increases the speed of the germination. Then, the propagation
85 step were done in the trays with peat moss as the medium and the shoots stem cutting were
86 left for two weeks for the development of the root. Then, the plants were transferred into
87 standard polybag (16cm x 30cm) which was filled with top soil as the medium [11].

88

89 **2.3 Soil preparation**

90

91 The soil medium was obtained from Unit Herba, Taman Pertanian Universiti and top soil was
92 used as the medium for the *Persicaria minor* planting. The top soil was transferred into the
93 polybag until it occupied three- quarter of the polybag

94

95 **2.4 Synthesis and properties of Zinc-Oxide Nanoparticles (ZnO-NPs)**

96

97 The ZnO-NPs was synthesized chemically in the Laboratory of BioPhysics of Physics
98 Department, Faculty of Science UPM by using sol gel method. ZnO-NPs is characterized as
99 solid, white odorless powdery. In room temperature, 200ml of ethanol was added 0.2M of
100 zinc acetate dehydrate. Then, the mixture was stirred for two hours to obtain clear solution.
101 Then, 1.0 M sodium hydroxide (NaOH) was titrated into the mixture until the pH 9 is reach.
102 The milky white slurry was obtained from the titration. To allow homogenous mixing, the
103 white slurry was stirred for one hour more. The sample was left for 24 hours to allow the
104 complete hydrolysis and gelation. The sample was separated from its solution. Then, the
105 sample was filtrated to obtain white precipitate. The sample is dried in an oven for 48 hours
106 at 100°C. The dried sample was grinded by mortar and pestle to yield ZnO powder.

107

108 **2.5 Experimental design**

109

110 A Completely Randomized Block Design (RCBD) was used in this study with three
111 replication. Each unit was consisted with eight plants and the totals of 96 plants were used in
112 this study. After a month, *Persicaria minor* was exposed to four different concentration of
113 zinc oxide nanoparticles (ZnO-NPs) which were (50,100 and 150 mg/L) and 0 mg/L as a
114 control. The ZnO-NPs was dissolved in distilled water before being applied to plants. 40 mL
115 of ZnO-NPs solution was applied to each plant.

116

117 **2.6 Plant maintenance**

118

119 The maintenance steps are very crucial to ensure the plant develops healthy and to avoid
120 the plants from wilting or attacked by any disease that can cause the plants to die. At the
121 early phase of cultivation, *Persicaria minor* were watered two times daily. The watering was
122 unnecessary only when the heavy rain occur. This to avoid the over watering to the plants
123 that can interfere the plants growth. The common insects that can interfere the plants growth
124 were removed quickly from the planting area.

125

126 **2.7 Collection of data**

127

128 The growth data collection was conducted once a week after the application of treatment for
129 the plants growth parameter. The destructive analysis and leaf gas exchange of the
130 experiment were conducted at the end of the experiment.

131

132 **2.7.1 Plant growth measurements**

133

134 The plant growth measurements were conducted to obtain data about height, number of leaf
135 and stem, diameter of stem, root to shoot ratio and the chlorophyll content.

136

137 **2.7.1.1 Plant height**

138

139 The plant height was measured starting from the stem on the soil surface until the highest
140 shoot growth using measuring tape.

141

142 **2.7.1.2 Plant basal diameter**

143

144 The plant basal diameter was measured by using vernier caliper at the tips of the plants.

145

146 **2.7.1.3 Plant leaves number**

147

148 The leaves of the *Persicaria minor* were counted manually in every three weeks.

149

150 **2.7.1.4 Chlorophyll content measurement**

151 The total chlorophyll content of the leaves were measured by using chlorophyll meter (SPAD
152 502). The leaves of the plants in each treatment for each replication were clipped by
153 chlorophyll meter clipper to obtain the reading.

154

155 **2.7.1.5 Plant fresh weight measurement**

156

157 The plants were removed first from the soil and all the dirt were removed under the flowing
158 tap water. Then, the shoot and the root parts were separated for further analysis and all the
159 plants parts were weighted separately using analytical balance.

160

161 **2.7.1.6 Dry weight (biomass) measurement**

162

163 The plants were dried in the oven at 60°C for 48 hours. Then, the measurements were
164 recorded by using electronic weighing scale.

165

166

167

168 **2.7.1.7 Root to shoot ratio**

169

170 The root to shoot ratio was determined by dividing the weight of the roots part to the shoot
171 part after the oven dried process.

172

173 **3.7.1.8 Plant leaf temperature determination**

174

175 The Infrared (IR) Thermometer was used to measure the plant leaves temperature.

176

177 **2.7.2 Leaf gas exchange measurement**

178

179 LI-6400XT (Li-COR Inc; Nebraska; USA) portable photosynthesis system was used to
180 measure the leaf gas exchange. This equipment was warmed and was calibrated with ZERO
181 IRGA mode for 30 minutes. The measurement was set at optimum condition which were 400
182 $\mu\text{mol mol}^{-1} \text{CO}_2$, 30°C cuvette temperature, 60% relative humidity with the rate of air flow
183 set at 500 $\text{cm}^3 \text{min}^{-1}$ and then the cuvette condition was modified at 800 $\mu\text{molm}^{-2}\text{s}^{-1}$
184 photosynthetically photon flux density (PPFD). The measurement process of gas exchange
185 was carried out between 9.00 am to 11.00 am by using fully expanded young leaves that
186 give the measurement of net photosynthesis (A), stomata conductance (gs) and transpiration
187 rate (E). Water use efficiency (WUE) was measured by using the formula of net
188 photosynthesis dividing with transpiration rate. This is automatic operation and the results
189 were saved in the the LI-6400XT console and Photosyn Assistant Software (Dundee
190 Scientific, Dundee, UK) was used to analyze it. Precautions were taken to avoid mistakes
191 during taking the measurements.

192

193 **2.7.3 Total Phenolics and Flavonoids Quantification**

194

195 Firstly, grounded plant tissue samples (0.1g) were extracted with 80% ethanol (10mL) on an
196 orbital shaker for 120 minutes at 50°C. The mixture was filtered and the filtrate was used for
197 the measurement of total phenolics and total flavonoids content. The total phenolic content
198 in the leaves sample was measured by the Follin-Ciocalteu reagent (SigmaAldrich, Missouri,
199 USA; diluted 10-fold). The absorbance was measured at 725 nm. The data were expressed
200 as mg g^{-1} gallic acid equivalent (mg GAE g^{-1} dry sample). The determination process of
201 total falvonoids content was measured by mixing a sample (1 mL) with NaNO_3 (Sigma
202 Aldrich, Missouri, USA; 0.3 mL) in a test tube that covered with aluminium foil. The mixture
203 then was left for 5 minutes. Then, 10% AlCl_3 (Wako Pure Chemical Industries Ltd; Tokyo,
204 Japan; 0.3 mL) was added followed by the addition of 1.0 NaOH (Kanto Chemical Co. Inc.;
205 Hokkaido, Japan; 2 mL). Then, the absorbance was measured at 510 nm using a
206 spectrophotometer with rutting as a standard (data were expressed as mg g^{-1} rutting dry
207 sample).

208

209 **2.7.4 Chlorophyll fluorescence determination**

210

211 The chlorophyll fluorometer was used to measure the chlorophyll florescence of the
212 *Persicaria minor*. The mature leaf tissue was obtained from the *Persicaria minor* plant that
213 cultivated at 20°C in glasshouse exposed with artificial light to give minimum photon flux
214 density of 550 $\mu\text{mol- m}^{-2} \text{S}^{-1}$ for 16 h photoperiod and phothosynthetically active radiation
215 were supplied at 250 $\mu\text{mol- m}^{-2} \text{S}^{-1}$ during 16 h photoperiod.

216

217

218

219

220

221
222
223
224
225
226
227
228
229
230
231
232
233
234
235
236
237
238
239
240
241
242
243
244
245
246
247
248
249
250
251
252
253
254
255
256
257
258
259
260
261
262
263
264
265
266
267
268
269
270
271
272
273

2.8 Statistical Analysis

Statistical Package for Social Sciences (SPSS) version 24 was used to analyze the recorded data. A two-way ANOVA Test was conducted to analyze data for all the parameters used in the experiment. Results were significant if the p-value level ≤ 0.05 .

3. RESULTS AND DISCUSSION

3.1 Plant Height

Fig.1 depicted the effect of three months treatment of zinc oxide nanoparticles (ZnO-NPs) on the plant height of *Persicaria minor*. The result from analysis of variance showed that there was a significant effect between different concentration of zinc oxide nanoparticles treatment toward the height of *Persicaria minor* ($P=0.05$). From the figure, the increasing of concentration of zinc oxide nanoparticles treatment increased the plant height of *Persicaria minor*. The highest plant height was recorded in 100 mg/L on 12 weeks after treatment with mean 26.017 cm that might indicates the optimum concentration for the plant. Meanwhile, the control treatment recorded the shortest plant height with mean 22.083 cm.

The appropriate concentration of zinc oxide nanoparticles plays significant role in plant growth and promotion [12]. From the result, on twelfth week after harvesting, the plants treated with zinc oxide nanoparticles have higher plant height as compared to plants in control treatment. This finding indicates that the application of zinc oxide nanoparticles can induced the growth of the plants. Kouhi et al. [13] explained that the zinc oxide nanoparticles possess plant growth promoting effects and were used as micronutrient fertilizer as the presence of these nanoparticles triggered the physiological processes, acting as growth regulating compound that increased the plant growth such as the plant height and biomass. In addition, Prasad et al. [14] also supported that zinc oxide nanoparticles possess beneficial effects in enhancing plant growth and development. The presence of zinc can enhance the biochemical, physiological and anatomical responds of the plants thus increased the plant growth such as plant height and biomass [15]. Therefore, it can be concluded that the zinc oxide nanoparticles treatment induced the plant growth and 100 mg/L can be considered as the best concentration in promoting the height of *Persicaria minor* plant.

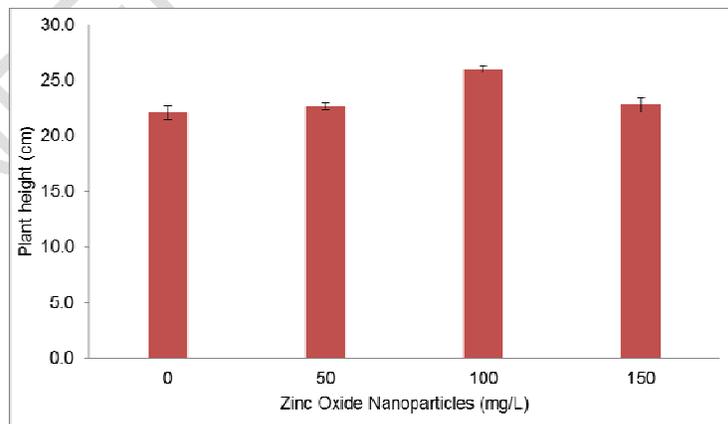


Fig.1. The effect of zinc oxide nanoparticles treatment (0, 50, 100 and 150) mg/L on plant height of *Persicaria minor*. Data are means with standard error of mean (SEM) of 24 replicates.

274
275
276
277
278
279
280
281
282
283
284
285
286
287
288
289
290
291
292
293
294
295
296
297
298
299
300
301
302
303
304
305
306
307
308
309
310
311
312
313
314
315
316
317
318
319
320
321
322
323
324
325
326

3.2 Plant Leaf Temperature

Fig. 2 highlighted the effect of three months treatment of zinc oxide nanoparticles (ZnO-NPs) on the total leaf temperature of *Persicaria minor*. The result from the analysis variance showed that there was a significant effect between different concentration of zinc oxide nanoparticles treatment toward the total leaf temperature of *Persicaria minor* ($P=0.05$). Based on the figure, the trend shows that the plant leaf temperature increased linearly with the concentration of zinc oxide nanoparticles treatment.

The plants maintaining their most important physiological process (photosynthesis) by maintaining their average leaf temperature at around 21 degree celsius [16]. The plants leaf temperature depends on the stomatal conductance and transpiration rates of the plants [17]. Transpiration is one of the best mechanism used by plants to cool themselves by 'pumping' out water from leaves through stomata [18]. From this study, the increasing of plant leaves temperature can be explained through the reduction of the stomatal conductance and transpiration rates of the plants due to the increasing the concentration of the treatment. This high temperature in turns will give negative effect to the photosynthesis process thus affect the plant yields. Therefore, it can be concluded that the zinc oxide nanoparticles treatment increased the plant leaf temperature due to the reduction of stomatal conductance and also transpiration rate of *Persicaria minor*.

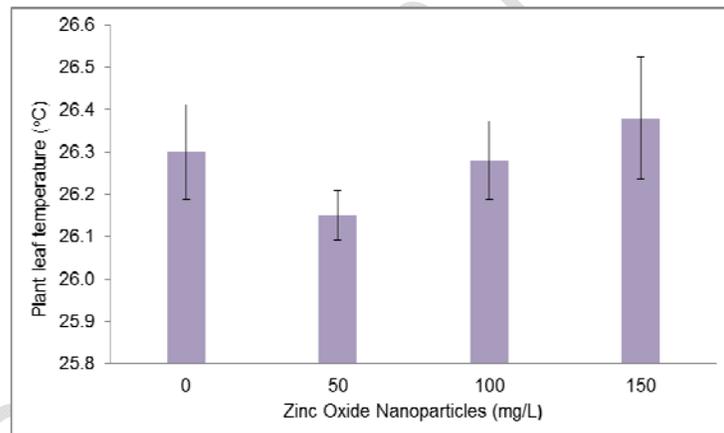
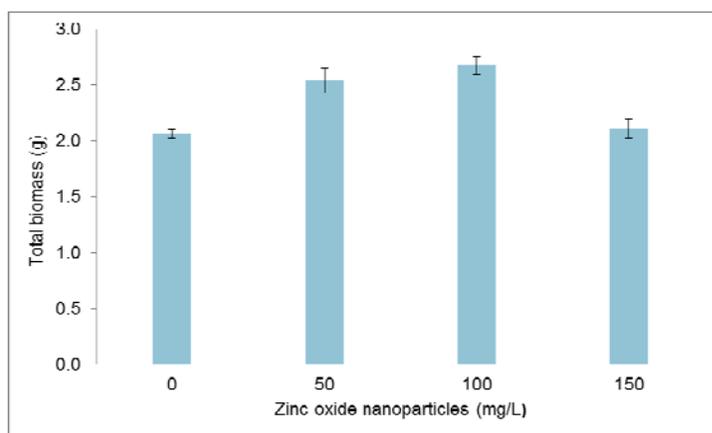


Fig.2.The effect of zinc oxide nanoparticles treatment (0, 50, 100 and 150) mg/L on plant leaf temperature of *Persicaria minor*. Data are means with standard error of mean (SEM) of 24 replicates.

3.3 Total Biomass

Fig.3 shows the effect of three months treatment of zinc oxide nanoparticles (ZnO-NPs) on the total biomass of *Persicaria minor*. The result from the analysis variance showed that there was a significant effect between different concentration of zinc oxide nanoparticles treatment toward the total biomass of *Persicaria minor* ($P=0.05$). From the result, it showed that higher concentration of zinc oxide nanoparticles increased the total biomass of *Persicaria minor* as plants treated with zinc oxide nanoparticles treatment have higher total biomass as compared to the control treatment and 100 mg/L have the highest value of total biomass with mean 2.675g.

327 The highest value of total biomass indicates that 100 mg/L was the optimum concentration of
328 zinc oxide nanoparticles for *Persicaria minor*. This study showed that the treatment of zinc
329 oxide nanoparticles increased the plant biomass so the treatment might be effective in
330 boosting the plant growth and yield. Similar finding was observed from study conducted by
331 Venkatachalam et al. [19] that revealed total biomass significantly increased in the zinc oxide
332 nanoparticles treated plants as compared to control. This is supported by Munir et al. [20]
333 that stated the treatment of zinc oxide nanoparticles increased the shoot and root dry weight
334 thus increased the plant biomass. The presence of zinc can enhanced the biochemical,
335 physiological and anatomical responds of the plants thus increased the plant growth such as
336 plant height and biomass [15]. Hence, it can be concluded that the presence of zinc oxide
337 nanoparticles can boost the *Persicaria minor* growth resulting in increasing of the plant total
338 biomass.



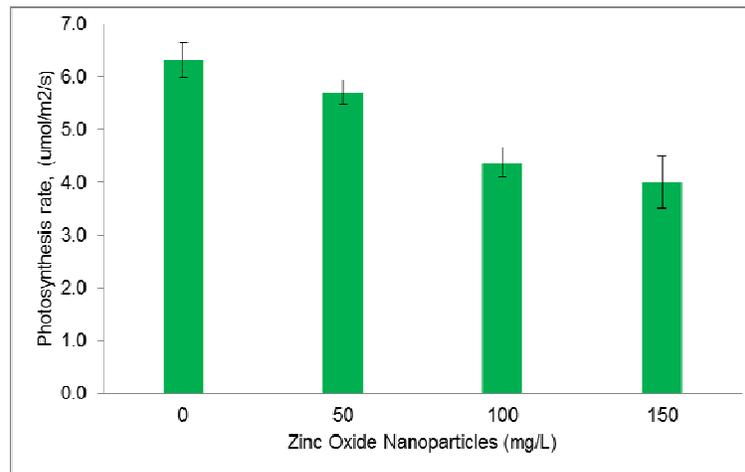
341
342
343
344
345
346
347
348
349
350
351
352
353
354
355 **Fig.3.The effect of zinc oxide nanoparticles treatment (0, 50, 100 and 150) mg/L on**
356 **total biomass of *Persicaria minor*. Data are means with standard error of mean (SEM)**
357 **of 24 replicates.**

358 3.4 Net photosynthesis rate (A)

359
360
361 Fig.4 illustrated the effect of three months treatment of zinc oxide nanoparticles (ZnO-NPs)
362 on the net photosynthesis rate of *Persicaria minor*. The exposure of zinc oxide nanoparticles
363 toward the *Persicaria minor* was highly and significantly affected the net photosynthesis rate
364 of the plant ($P=0.05$). From the result, the highest photosynthesis rate was recorded in
365 control treatment while the lowest photosynthesis rate was recorded in 150 mg/L of zinc
366 oxide nanoparticles treatment with mean 6.32 and 4 respectively. From the figure, the
367 photosynthesis rate of the plant reduced with the increasing concentration of zinc oxide
368 nanoparticles treatment.

369
370 Photosynthesis is the perfect measurement to access plant performance. From this study,
371 the net photosynthesis of *Persicaria minor* was reduced with the increasing concentration of
372 zinc oxide nanoparticles treatment. Photosynthesis is highly affected in plants that exposed
373 to excess heavy metal where higher level of zinc oxide nanoparticles inhibits the
374 photosynthetic apparatus and caused critical changes to chlorophyll structure and amount
375 [21]. This finding is also similar with Wang et al. [22] that revealed the presence of zinc oxide
376 nanoparticles reduced the chlorophyll content in leaves thus reduced the photosynthetic
377 efficiency in plants. In addition, plants exposed to high concentration of zinc oxide
378 nanoparticles have low photosynthetic efficiency due to the reduction of chlorophyll content
379 and also damaged to the photochemical system. Therefore, it can be concluded that the

380 presence of zinc oxide nanoparticles reduced the photosynthetic efficiency of *Persicaria*
381 *minor* plants.
382



383
384
385
386
387
388
389
390
391
392
393
394
395
396
397
398
399
400 **Fig.4: The effect of zinc oxide nanoparticles treatment (0, 50, 100 and 150) mg/L net**
401 **photosynthesis rate of *Persicaria minor*. Data are means with standard error of mean**
402 **(SEM) of 24 replicates.**

403 404 **3.5 Transpiration rate (E)**

405
406 Fig.5 depicted the effect of three months treatment of zinc oxide nanoparticles (ZnO-NPs) on
407 the transpiration rate of *Persicaria minor*. The exposure of different concentration of zinc
408 oxide nanoparticles toward the *Persicaria minor* was highly and significantly affected the
409 transpiration rate of the plant ($P=0.05$). From the figure, the transpiration rate of the plant
410 reduced with the increasing concentration of zinc oxide nanoparticles treatment. The
411 transpiration rate of 50 mg/L treatment was significantly higher with mean 2.342 while the
412 lowest transpiration rate of *Persicaria minor* was observed in 150 mg/L of zinc oxide
413 nanoparticles treatment with mean 1.42.
414

415 Transpiration is a process of the movement of water through plant and this process mainly
416 take place in leaves. Transpiration process is mainly controlled by the opening and closing of
417 the stomata. From the result, the transpiration rate reduced with the increasing concentration
418 of zinc oxide nanoparticles treatment. This finding is similar with Xiaoping et al. [23] that
419 revealed both transpiration rate and stomatal conductance reduced in plants treated with
420 zinc oxide nanoparticles. The stomatal closure reduced the transpiration rate of the plants.
421 According to Vankova et al. [24], the presence of zinc oxide nanoparticles induced the
422 production of plant stress hormone, abscisic acid and this hormone mainly accumulated in
423 leaves. The higher level of abscisic acid triggered the stomatal closure which in turn reduced
424 the transpiration rate in plants. Hence, the application of zinc oxide nanoparticles reduced
425 transpiration rate of *Persicaria minor* due to the high level of stress hormone that cause the
426 closure of stomata.
427

428
429
430
431
432

433
434
435
436
437
438
439
440
441
442
443
444
445
446
447
448
449
450
451
452
453
454
455
456
457
458
459
460
461
462
463
464
465
466
467
468
469
470
471
472
473
474
475
476
477
478
479
480
481
482
483
484
485

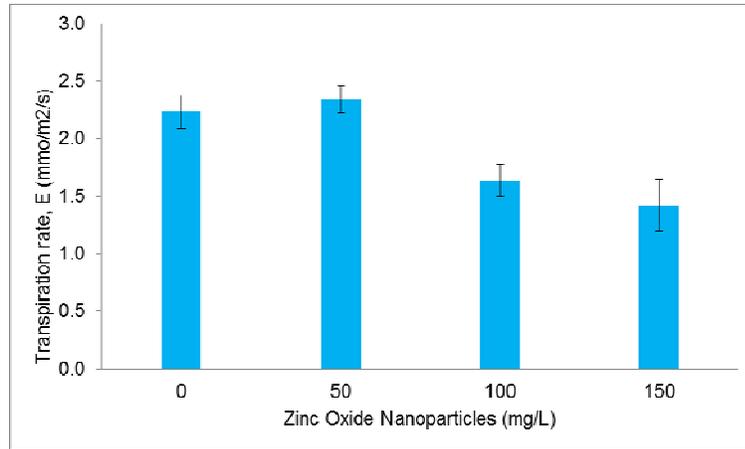
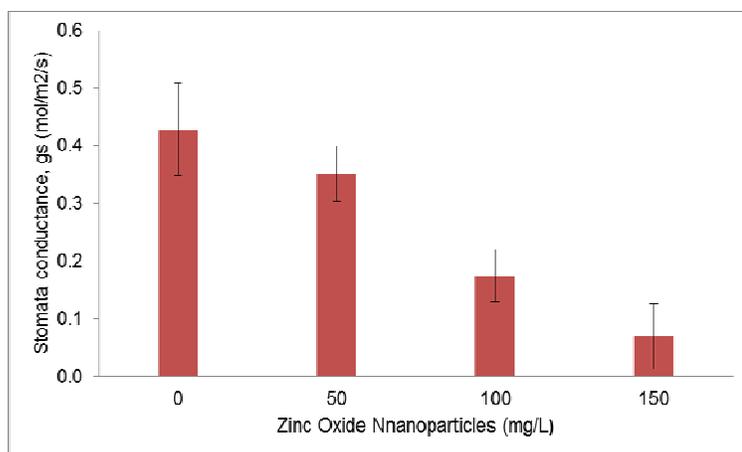


Fig.5. The effect of zinc oxide nanoparticles treatment (0, 50, 100 and 150) mg/L transpiration rate of *Persicaria minor*. Data are means with standard error of mean (SEM) of 24 replicates.

3.6 Stomatal conductance (gs)

Fig.6 shows the effect of three months treatment of zinc oxide nanoparticles (ZnO-NPs) on the transpiration rate of *Persicaria minor*. The exposure of different concentration of zinc oxide nanoparticles toward the *Polygonum minus* was highly and significantly affected the stomatal conductance of the plant ($P=0.05$). From the figure, the stomatal conductance of the plant reduced as the concentration of the zinc oxide nanoparticles treatment increasing. The highest stomatal conductance was recorded in control treatment while the lowest stomatal conductance was observed in 150 mg/L of zinc oxide nanoparticles treatment with mean 0.428 and 0.07 respectively.

Stomatal conductance is a measure of the degree of stomatal opening and a good indicator in accessing plant water status [25]. The finding showed reduction in stomatal conductance due to the increasing of zinc oxide nanoparticles concentration is similar with Xiaoping et al. [23] that proved the higher concentration of zinc oxide nanoparticles reduced the stomatal conductance resulting in low photosynthetic efficiency of the plants. Singh and Bhati [26] also stated that high amounts of zinc oxide nanoparticles can restrict the stomatal conductance. This might due to the toxicity of the treatment disturbed the cell mechanism thus alters the stomatal function. Tsonev and Lidon [21] explained that the stomatal response to high concentration of zinc oxide nanoparticles is related to the changes in carbonic anhydrase (CA) activity. Carbonic anhydrase is enzyme that responsible for the stomatal activity and the presence of zinc oxide nanoparticles influenced the CA activity that triggered the stomatal closure thus reduced the stomatal conductance of the plants. Hence, it can be concluded that the presence of zinc oxide nanoparticles alter the stomatal mechanism thus reducing the stomatal conductance of *Persicaria minor* plants.



486
487
488
489
490
491

Fig.6. The effect of zinc oxide nanoparticles treatment (0, 50, 100 and 150) mg/L on stomatal conductance of *Persicaria minor*. Data are means with standard error of mean (SEM) of 24 replicates.

492
493

3.7 Maximum efficiency of photosystem II

494
495
496
497
498
499
500
501
502
503

Fig.7 depicted the effect of three months treatment of zinc oxide nanoparticles (ZnO-NPs) on the maximum efficiency of photosystem II of *Persicaria minor*. The exposure of different concentration of zinc oxide nanoparticles toward the *Persicaria minor* was highly and significantly affected the maximum efficiency of photosystem II of the plant ($P=0.05$). Based on the figure, increasing the concentration of zinc oxide nanoparticles caused the maximum efficiency of photosystem II to decrease. The highest value of maximum efficiency of photosystem II was observed in control treatment while the lowest value of maximum efficiency of photosystem II was recorded in 150 mg/L treatment with mean 0.758 and 0.522 respectively.

504
505
506
507
508
509
510
511
512
513
514
515
516
517
518
519
520
521
522
523
524
525

The phytotoxicity of zinc oxide nanoparticles can be accessed through the efficiency of photosynthetic mechanism (chlorophyll florescence) that act as indicator in phytotoxicity assays. The finding of this study revealed that increasing the zinc oxide nanoparticles concentration resulting in lower maximum efficiency of photosystem II of *Polygonum minus*. Wang et al. [22] stated that the treatment of zinc oxide nanoparticles reduced the chlorophyll florescence parameter and damaged the photochemical system. This finding can be explained further that the presence of zinc oxide nanoparticles induced the oxidative stress in plants and increase the production of reactive oxygen species (ROS) which alter the gene expression pathway thus reduced the chlorophyll florescence in plants. Therefore, it can be concluded that the zinc oxide nanoparticles treatment reduced the chlorophyll florescence parameters of *Persicaria minor* plants.

526
527
528
529
530
531
532
533
534
535
536
537
538
539
540
541
542
543
544
545
546
547
548
549
550
551
552
553
554
555
556
557
558
559
560
561
562
563
564
565
566
567
568
569
570
571
572
573
574
575
576
577
578

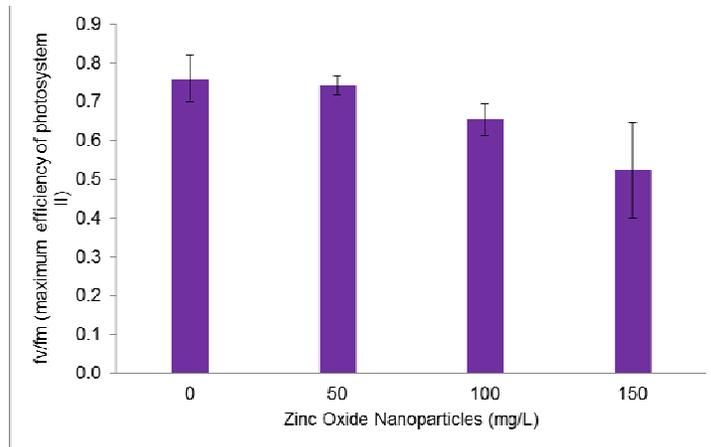


Fig.7. The effect of zinc oxide nanoparticles treatment (0, 50, 100 and 150) mg/L on maximum efficiency of photosystem II of *Persicaria minor*. Data are means with standard error of mean (SEM) of 24 replicates.

3.8 Maximum yield of photosystem II

Fig.8 shows the effect of three months treatment of zinc oxide nanoparticles (ZnO-NPs) on the maximum yield of photosystem II of *Persicaria minor*. The exposure of different concentration of zinc oxide nanoparticles toward the *Persicaria minor* was highly and significantly affected the maximum yield of photosystem II of the plant ($P=0.05$). The trend shows that increasing the concentration of zinc oxide nanoparticles caused the maximum yield of photosystem II to decrease. Based on the figure, 50 mg/L of zinc oxide nanoparticles treatment shows the highest value of maximum yield of photosystem II with mean 3.166 when compared with other treatment.

The photosynthetic pigments (chlorophyll fluorescence) act as indicator in phytotoxicity assays in accessing the phytotoxicity of zinc oxide nanoparticles towards the plant. From this study, it was observed that the treatment of zinc oxide nanoparticles reduced the maximum efficiency of photosystem II which in turn reduced the maximum yield of photosystem II of *Persicaria minor*. According to Tsonev and Lidon [21], inside the chloroplast lamellae, the presence of zinc oxide nanoparticles caused the inhibition of photosynthetic electron transport and implicates the water evolving complex of photosystem II thus inhibits the photolysis and oxygen emission that disturb the conformation of photosystem II core complex. This mechanism explained how the zinc oxide nanoparticles treatment reduced the efficiency and yield of photosystem II in plants. Hence, the treatment of zinc oxide nanoparticles reduced the maximum yield and efficiency of photosystem II which in turn disturbed the photosynthetic process of *Persicaria minor* plant.

579
580
581
582
583
584
585
586
587
588
589
590
591
592
593
594
595
596
597
598
599
600
601
602
603
604
605
606
607
608
609
610
611
612
613
614
615
616
617
618
619
620
621
622
623
624
625
626
627
628
629
630
631

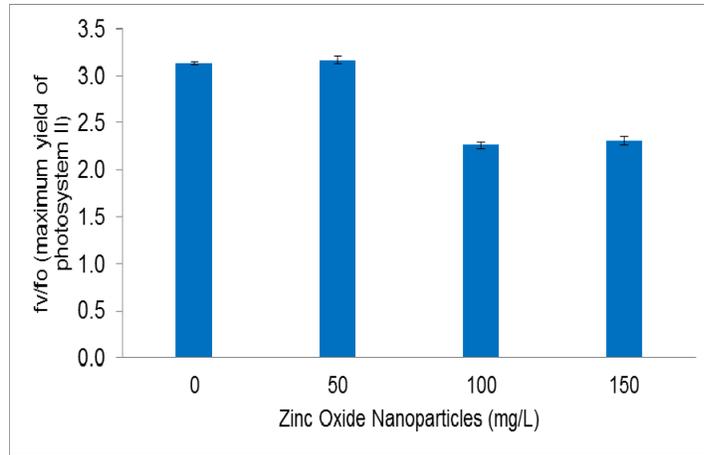


Fig.8. The effect of zinc oxide nanoparticles treatment (0, 50, 100 and 150) mg/L on maximum yield of photosystem II of *Persicaria minor*. Data are means with standard error of mean (SEM) of 24 replicates.

3.9 Minimal fluorescence

Fig.9 shows the effect of three months treatment of zinc oxide nanoparticles (ZnO-NPs) on the minimal fluorescence of *Persicaria minor*. The exposure of different concentration of zinc oxide nanoparticles toward the *Polygonum minus* was highly and significantly affected the maximum yield of photosystem II of the plant ($P=0.05$). The result showed that higher concentration of zinc oxide nanoparticles treatment resulted in higher value of minimal fluorescence of *Persicaria minor*. The highest value of minimal inflorescence was recorded in 150 mg/L with mean 627.2 while the lowest value was observed in 50 mg/L with mean 462.6.

Higher minimal fluorescence indicates higher heat dissipation of plants. This might due to the presence of zinc oxide nanoparticles that induced stress in plants thus caused plants to produce high amount of heat. From this study, the treatment of zinc oxide nanoparticles reduced the transpiration rate of *Persicaria minor*. This reduction might related with the increasing of minimal fluorescence of the plants. The high minimal fluorescence can cause heat stress to the plants. Heat stress is defined as the increase temperature beyond the threshold level that cause damage to plant growth and development [27]. Therefore, it can be deduced that the treatment of zinc oxide nanoparticles increased the minimal fluorescence of *Persicaria minor* due to the reduction in transpiration rate of the plants.

632
633
634
635
636
637
638
639
640
641
642
643
644
645
646
647
648
649
650
651
652
653
654
655
656
657
658
659
660
661
662
663
664
665
666
667
668
669
670
671
672
673
674
675
676
677
678
679
680
681
682
683
684

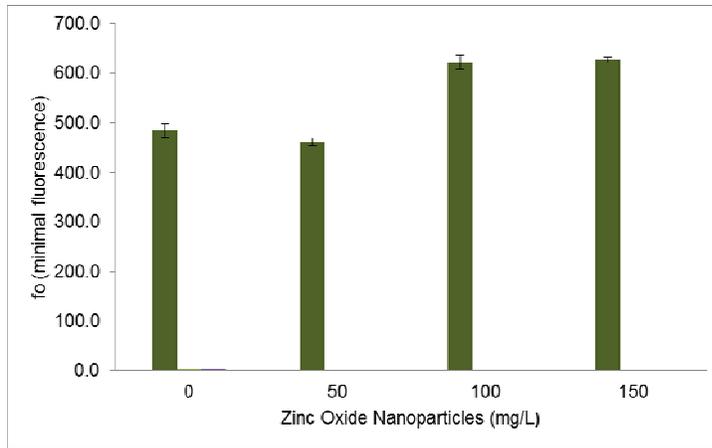


Fig.9. The effect of zinc oxide nanoparticles treatment (0, 50, 100 and 150) mg/L on minimal fluorescence of *Persicaria minor*. Data are means with standard error of mean (SEM) of 24 replicates.

3.10 Performance index (PI)

Fig.10 indicated the effect of three months treatment of zinc oxide nanoparticles (ZnO-NPs) on the performance index of *Persicaria minor*. The exposure of different concentration of zinc oxide nanoparticles toward the *Polygonum minus* was highly and significantly affected the performance index of the plant ($P=0.05$). Based on the figure, the highest and lowest performance index were observed in control treatment and 150 mg/L of zinc oxide nanoparticles treatment with mean 2.422 and 1.504 respectively. The performance index of *Persicaria minor* reduced when treated with higher concentration of zinc oxide nanoparticles that indicates zinc oxide nanoparticles increased the plant stress.

Nanoparticles such as zinc oxide and silver were located on the surface of plants cells and induced the oxidative stress to the cells by the activation of oxidative stress signaling [28]. From this study, it was observed that the treatment of zinc oxide nanoparticles reduced the plants performance index. Zahed et al. [28] stated that the generation of reactive oxygen species (ROS) due to the zinc oxide nanoparticles treatment alter the gene expression and cell mechanism which in turn reduced the performance index of the plants. Wang et al. [22] explained that the toxicity of zinc oxide nanoparticles reduced chlorophyll content plants, resulted in low photosynthesis efficiency thus reduced the plants performance. Hence, it can be concluded that the presence of zinc oxide nanoparticles induced stress in *Persicaria minor* resulting in low performance index of the plants.

685
686
687
688
689
690
691
692
693
694
695
696
697
698
699
700
701
702
703
704
705
706
707
708
709
710
711
712
713
714
715
716
717
718
719
720
721
722
723
724
725
726
727
728
729
730
731
732
733
734
735
736
737

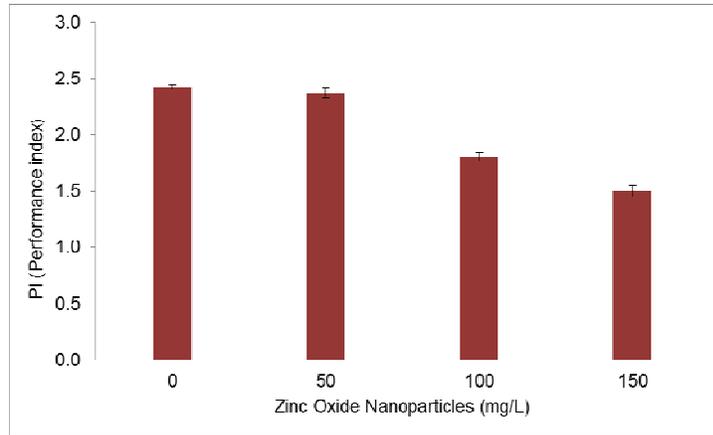


Fig.10. The effect of zinc oxide nanoparticles treatment (0, 50, 100 and 150) mg/L on performance index of *Persicaria minor*. Data are means with standard error of mean (SEM) of 24 replicates.

3.11 Total phenolics content

Fig.11 shows the effect of three months treatment of zinc oxide nanoparticles (ZnO-NPs) on the total phenolics production of *Persicaria minor*. The exposure of different concentration of zinc oxide nanoparticles toward the *Polygonum minus* was highly and significantly affected the total phenolics production of the plant ($P=0.05$). Based on the figure, the total phenolics production of the plant was directly proportional with the concentration of zinc oxide nanoparticles treatment. The lowest total phenolics production was recorded in control treatment while the highest total phenolics production was recorded in 150 mg/L treatment with mean 1.444 and 3.82 respectively. This result indicates that zinc oxide nanoparticles treatment induced stress and increased the secondary metabolites production of *Persicaria minor*.

Phenolics are compound that produced by plants to protect plants against stress. These compound play significant role in plant development (lignin and pigment biosynthesis) and also provided structural integrity for plant's support [29]. From this study, the greater production of total phenolics content in *Polygonum minus* with the increasing of concentration treatment revealed that the presence of zinc oxide nanoparticles induced stress towards the plants. This finding is supported by Rastogi et al. [30] that stated zinc oxide nanoparticles treatment induced the Reactive Oxygen Species (ROS) production in plants thus increased plants stress. They also stated that higher concentration of zinc oxide nanoparticles lead to the damage of plant cell wall and plasma membrane thus induced the production of plant secondary metabolites for plants defense against disease and threat. Therefore, it can be concluded that higher concentration of zinc oxide nanoparticles lead to plant stress and boost the plants secondary metabolites production which in turn can enhance the defense response of *Persicaria minor* plants.

738
739
740
741
742
743
744
745
746
747
748
749
750
751
752
753
754
755
756
757
758
759
760
761
762
763
764
765
766
767
768
769
770
771
772
773
774
775
776
777
778
779
780
781
782
783
784
785
786
787
788
789
790

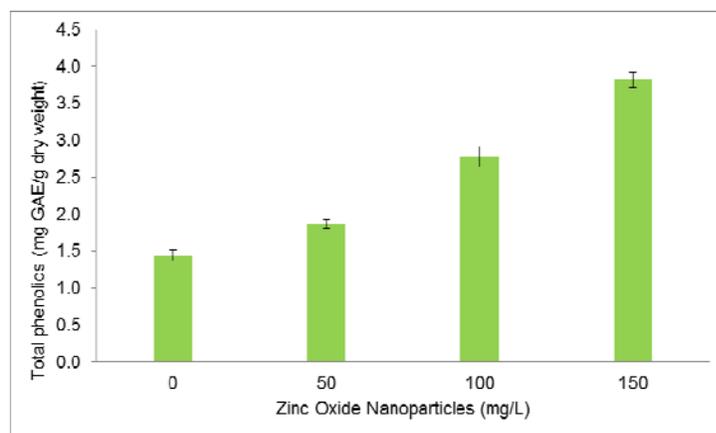


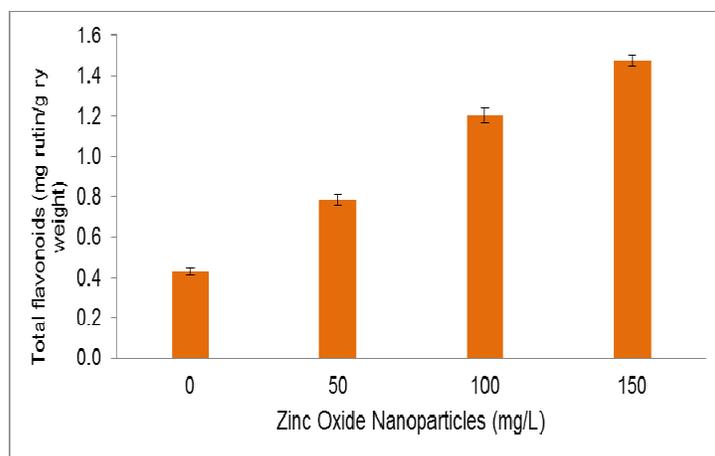
Fig.11. The effect of zinc oxide nanoparticles treatment (0, 50, 100 and 150) mg/L on total phenolics production of *Persicaria minor*. Data are means with standard error of mean (SEM) of 24 replicates

3.12 Total flavonoids content

Fig.12 shows the effect of three months treatment of zinc oxide nanoparticles (ZnO-NPs) on the total flavonoids production of *Persicaria minor*. The exposure of different concentration of zinc oxide nanoparticles toward the *Persicaria minor* was highly and significantly affected the total flavonoids production of the plant ($P=0.05$). Based on the figure, the total flavonoids production of the plant was directly proportional with the concentration of zinc oxide nanoparticles treatment. The lowest total flavonoids production was recorded in control treatment while the highest total flavonoids production was recorded in 150 mg/L treatment with mean 0.43 and 1.478 respectively. This result indicates that zinc oxide nanoparticles treatment induced stress and increased the secondary metabolites production of *Persicaria minor*.

Flavonoids are a wide group of plants chemicals (phytonutrients) that found mostly in fruits and vegetables. Flavonoids plays significant role in pharmacological field since this compound is a good source of antioxidant and anti-inflammatory, protect skin, enhanced brain function and also good for blood pressure regulation [31]. From this study, the greater production of total flavonoids content in *Polygonum minus* with the increasing of concentration treatment revealed that the presence of zinc oxide nanoparticles induced stress towards the plants. This finding is similar with Zafar et al. [32] the higher treatment concentration of zinc oxide nanoparticles generates oxidative stress of plants thus increasing the plant secondary metabolites production to protect plants against stress. The initial response of plants towards the presence of nanoparticles involved the increasing level of reactive oxygen species (ROS), cytoplasmic Ca^{2+} and up regulation of nitrogen activated protein kinase (MAPK) cascades thus activates the plants secondary metabolites that act against stress to protect the plants [33]. In addition, the presence of zinc oxide nanoparticles enhanced the expression of genes related to antioxidant capacity thus boost the defense mechanism of the plants by enhancing the production of plants secondary metabolites. Hence, it can be concluded that the presence of zinc oxide nanoparticles enhanced the *Persicaria minor* secondary metabolites production by increasing the total flavonoids production of the plants.

791
792
793
794
795
796
797
798
799
800
801
802
803
804
805



806 **Fig.12. The effect of zinc oxide nanoparticles treatment (0, 50, 100 and 150) mg/L on**
807 **total flavonoids production of *Persicaria minor*. Data are means with standard error of**
808 **mean (SEM) of 24 replicates.**

810 **4. CONCLUSION**

811
812
813
814
815
816
817
818
819
820
821
822

Overall, the treatment of zinc oxide nanoparticles increased the growth parameter of the plants as the treated plants showed higher value of plant height and total biomass when compared to plants in control treatment. However, the treatment of zinc oxide nanoparticles reduced the photosynthesis rate, transpiration rate and stomatal conductance thus reduced the performance index of *Persicaria minor* plants. The treatment also might induced the plants stress as it was significantly observed that the production of secondary metabolites (total phenolics and flavonoids production) were directly proportional with the treatment concentration that used mainly for plants production against stress. From this study, it can be concluded that the optimum concentration of zinc oxide nanoparticles for enhancing the *Persicaria minor* growth was 100 mg/L because it recorded the highest value for most of the plants growth parameters.

824 **5. REFERENCES**

825
826
827
828
829
830
831
832
833
834
835
836
837
838
839
840
841
842
843

1. National Health Portal. Introduction and Importance of Medicinal Plants and Herbs. 2016. Accessed 8 July 2018. Available: https://www.nhp.gov.in/introduction-and-importance-of-medicinal-plants-and-herbs_mtl.
2. Christopher PV, Parasuraman S, Christina JMA, Asmawi MZ, Vikneswaran M. Review on Polygonum minus. Huds, a commonly used food additive in Southeast Asia. Pharmacognosy Research. 2015; 7(1): 1–6.
3. Vikram P, Chiruvella KK, Husna IAR, Mohammed A. A recent review on phytochemical constituents and medicinal properties of kesum (Polygonum minus Huds.). Asian Pacific Journal of Tropical Biomedicine. 2014; 4(6): 430-435.
4. Mirzaei H, Darroudi M. Zinc oxide nanoparticles: Biological synthesis and biomedical applications. Journal of Ceramics International. 2016; 43(1): 907-914. doi: <https://doi.org/10.1016/j.ceramint.2016.10.051>.
5. Vishnu DR, Minkina T, Yaning C, Sushkova S, Chaplugin VA, Mandzhieva S. A review on salinity adaptation mechanism and characteristics of Populus euphratica, a boon for arid ecosystems. Acta Ecológica Sinica. 2016; 36(6): 497-503.
6. Sabir S, Arshad M, Sunbal KC. Zinc Oxide nanoparticles for revolutionizing agriculture: synthesis and applications. Scientific World Journal. 2014; 8. Doi:<https://dx.doi.org/10.1155%2F2014%2F925494>

- 844 7. Faizan M, Faraz A, Yusof M, Khan M, Hayat S. Zinc oxide nanoparticle-mediated
845 changes in photosynthetic efficiency and antioxidant system of tomato plants. Journal of
846 Springer. 2017; 56(2): 678-686. doi <https://doi.org/10.1007/s11099-017-0717-0>
- 847 8. Manzer HS, Waibi MH, Firoz M, Mutahhar YAK. Role of nanoparticles in plants. 2015.
848 Accessed 18 February 2018. Available: [file:///C:/Users/User/Downloads/9783319145013-](file:///C:/Users/User/Downloads/9783319145013-c2.pdf)
849 [c2.pdf](file:///C:/Users/User/Downloads/9783319145013-c2.pdf).
- 850 9. Biology Reference. Secondary Metabolites in Plants. 2018. Accessed 13 July 2018.
851 Available:[http://www.biologyreference.com/Re-Se/Secondary-Metabolites-in Plants.html](http://www.biologyreference.com/Re-Se/Secondary-Metabolites-in%20Plants.html).
- 852 10. Schater H, Wink M. Medicinally important secondary metabolites in recombinant
853 microorganisms or plants: progress in alkaloid biosynthesis. Journal of Biotechnol.
854 2009; 4(12): 1684-1703. doi: 10.1002/biot.200900229.
- 855 11. Ibrahim MH, Nurul NM, Zain NAM. Growth, carbon assimilation and biochemical changes
856 of *Polygonum minus* Huds. as affected by nitrogen fertilization. Journal of Annual
857 Research and Review in Biology. 2018; 26(1): 1-17.
- 858 12. Paripark R, Sutichai S, Sutee C. ZnO nanoparticles affect differently the morphological
859 and physiological responses of riceberry plants (*Oryza sativa* L.). SNRU Journal of
860 Science and Technology. 2018; 10(1): 75-81.
- 861 13. Kouhi SMM, Lahouti M, Ganjeali A, Entezari MH. Comparative phytotoxicity of ZnO
862 nanoparticles, ZnO microparticles and Zn²⁺ on rapeseed (*Brassica napus* L.):
863 investigating a wide range of concentration. Toxicology and Environmental Chemistry.
864 2014; 96(6): 861-868.
- 865 14. Prasad TNKV, Sudhakar Y, Sreenivasulu P, Latha V, Munaswamy K, Raja RJS,
866 Sreeprasad PR, Sajanlal Pradeep T. Effect of nanoscale zinc oxide nanoparticles on
867 germination, growth and yield of peanut. Journal of Plant Nutrition. 2012; 35: 905-927.
- 868 15. Vishnu DR, Tatiana MM, Arvind B, Svetlana NS, Saglar M, Ritu S, Andrey G, Viktoriia
869 ST, William OP, Karen AG, Hamshik SM. Effects of zinc-oxide nanoparticles on soil,
870 plants, animals and soil organisms: a review. Environmental Nanotechnology, Monitoring
871 and Management. 2018; 9: 76-84.
- 872 16. Ledford H. Leaves keep their cool. 2008. Accessed 13 November 2018. Available:
873 <https://www.nature.com/news/2008/080611/full/news.2008.884.html>.
- 874 17. Urban J, Miles WI, McGuire MA, Teskey RO. Increase in leaf temperature open stomata
875 and decouples net photosynthesis from stomatal conductance in *Pinus taeda* and
876 *Populus deltoids* x *nigra*. Journal of Experimental Botany. 2017; 68(7): 1757-1767.
- 877 18. Harlequin G. Plants in high temperature. 2016. Accessed 13 November 2018. Available:
878 <http://www.harlequingardens.com/mikls-articles/plants-in-high-temperatures/>.
- 879 19. Venkatachalam P, Priyanka N, Manikandan K, Ganeshbaru I, Indiraarulsevi P, Geetha
880 N, Muralikrishna K, Bhattacharya RC, Tiwari M, Sharma N, Sahi SV. Enhanced plant
881 growth promoting role of phycomolecules coated zinc oxide nanoparticles with P
882 supplementation in cotton (*Gossypium hirsutum* L.). Plant Physiology and
883 Biochemistry. 2017; 110: 118-127.
- 884 20. Munir T, Rizwan M, Kashif M, Shahzad A, Ali S, Amin N, Zahid R, Alam MFE, Imran M.
885 Effect of zinc oxide nanoparticles on the growth and zinc uptake in wheat
886 (*Triticum aestivum* L.) by seed priming method. Digest Journal of Nanomaterials and
887 Biostructures. 2018; 13(1): 315-32.
- 888 21. Tsonev T, Lidon FJC. Zinc in plants- an overview. American Journal Food Agriculture.
889 2012; 24(4): 322-333.
- 890 22. Wang XP, Li QQ, Pei ZM, Wang SC. Effects of zinc oxide nanoparticles on the growth,
891 photosynthetic traits and antioxidative enzymes in tomato plants. Biological Plantarum.
892 2018; 1-8.
- 893 23. Xiaoping W, Chen S, Xiyu Y, Shucai W. Zinc oxide nanoparticles affect the biomass
894 accumulation and photosynthesis in *Arabidopsis*. Frontiers Plant Science. 2016). doi
895 10.3389/fpls.2015.01243
- 896 24. Vankova R, Lamda P, Podlipna R, Dobrey PT, Prerostova S, Langjansova L, Gaudinova

- 897 A, Motkova K, Knirsch V, Vanek T. ZnO nanoparticles effects on hormonal pools in
898 Arabidopsis Thaliana. *Science Total Environment*. 2017; 593-594.
- 899 25. Gimenez C, Gallardo M, Thompson RB. Plant-water relations. *Earth System and*
900 *Environmental Science*. 2013; 231-238.
- 901 26. Singh G, Bhati M. Mineral toxicity and physiological functions in tree seedling irrigated
902 with effluents of varying chemistry in sandy soil of dry region. *Journal of Environmental*
903 *Science*. 2003; 21(1): 45-63.
- 904 27. Nahla SH, Taher ASED, Mohamed HH, Ibrahim HB, Asmaa AM. Magnetic and zinc oxide
905 nanoparticles alleviated heat stress in wheat plants. *Current Nanomaterials*. 2018; 3(1):
906 32-43.
- 907 28. Zahed H, Ghazala M, Komatsu S. Plant's responses to nanoparticles stress. *International*
908 *Journal of Molecular Science*. 2015; 16(11): 26644-26653.
- 909 29. Bhattacharya A, Sood P, Citovsky V. The roles of plant phenolics in defense and
910 communication during Agrobacterium and Rhizobium infection. *Molecular Plant*
911 *Pathology*. 2010; 11(5): 705-719.
- 912 30. Rastogi A, Zivcak M, Sytar O, Kalaji HM, Xiaolan H, Mbarki S, Brestic M. Impact of metal
913 and metal oxide nanoparticles on plant: a critical review. *Frontiers in Chemistry*. 2017; 5:
914 78.
- 915 31. Szalay S. What are flavonoids?. 2015. Accessed 13 November 2018.
916 <https://www.livescience.com/52524-flavonoids.html>.
- 917 32. Zafar H, Ali A, Ali JS, Haq IU, Zia M. Effect of ZnO nanoparticles on Brassica seedlings
918 and stem explants: growth dynamics and antioxidant response. *Frontiers Plants Science*.
919 2016; 7: 535.
- 920 33. Marslin G, Sheeba CJ, Franklin G. Nanoparticles alter secondary metabolism in plants
921 via ROS burst. *Frontiers in Plant Science*. 2017; 8: 832.
922