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Influence of Metionine and Broccoli Seed Extract (*Brassica oleracea L var Italica*) to Increasing Adventive Shoot and in Vitro Synthesis of Sulforaphane

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ABSTRACT

This study intended to produce the best combination of concentration on meitionin and broccolli seed extract due to multiplication of adventive shoot and the best combination in increasing in Vitro sulfroraphane. This research was conducted at Biotechnological Laboratory, Faculty of Agriculture, University of Sam Ratulangi, Manado of Indonesia. Analysis of sulfroraphane was carried out at Chemical Laboratory, Public Polythecnic, Malang of Indonesia during 4 months. The methodology consisted of Perfect Random Design which factorially arranged the data into 2 factors those were A, included metionine of 0 mg, 50 mg, 100 mg, 150 mg, and B, included extract of broccoli seed of 0 g, 1 g, 3 g. Each of treatments were returned for 4 times. Explant used in this research was adventive shoot as induction result of initial research that was combination of 1ppm of NAA and 5 ppm of BAP. This research used the media of Murashide and Skoog. Analysis of data was carried out using analysis of variance and then continued by BNT test of 5%.Observed variables were included number, fresh weight of shoot, height of shoot, leaves number and sulfroraphane content of adventive shoot. Result of variance analysis showed that there were interaction with significant difference between treatment of metionine and extract of broccoli seed due to their influence to the fresht weight of shoot, number of leaves and content of sulfroraphane. But the intercation was not significant difference between the treatment of metionine and extract of broccoli seed due to the indluence of the number and height of shoot. Hence, the teatment of metionine was significant different due to teh number and height of shoot. The treatment of broccoli seed extract was significant different due to only the height of shoot. Treatment on 2 g and 3 d of broccoli seed extract or 50 mg of metionine was the best influence to the number and wet weight of shoot, the number and height of shoot. Combination treatment on 100 mg of metionine and 1 g of broccoli seed extract produced the highest content of sulfrophane that was 182.09 ng/g of sprout material

Keywords: broccoli, metionine, extract of broccoli seed, NAA, BAP, increasing of sproud, sulfroraphane

INTRODUCTION

Vegetables type of cabbage was mentioned as the richest essence of antioxidane, either in number or type. The best compound of antioxydant saving in broccoli was sulforaphane. Beside that, there were betakaroten, indola, kuersetin, and glutation. *Brassica Oleracea L Var italica* or broccoli had content of fatty, protein, carbohydrate, fibre, water, essence of iron, calcium, mineral, and kind of vitamine like A, C, E, roboflamin, nikotanamide [1]. Some researches showed that natural food was rich of essence which was useful for healthy. Li *et.al*. [2] said that sulforaphane with 1-5 umol/l decreased aldehyde dehydrogenase and population of positive cells of cancer about 65-80% at human cancer cell ($P < 0.01$) and decreasing size and number of mammpsphere approximate 8-125 times and 45-75% ($P < 0.01$), especially for sulforaphane. He concluded that sulforaphane pursued cancer cell and regulated back the line of Wnt/β-catenin.

There were two types of antioxidant essence those were direct and indirect. Type of direct antioxidance was as a substance which helped the process of fisilogy, bio-chemical, and seluler like glutation, tocopherol, ascorbic acid, and caretenoid. Indirect antioxydant was as a substance which was not able to help radical or redox reaction but in cell, it had ability with any kind of mechanism to detokcificate and inducted the protect to animal and its cell for rejecting carsinogen and mutagenesys like glutathione transferees, NAD(P)H reductive, epoxide hydrolase, and heme oxygenizes which was functioned in inducer of phase II [3]. There was said that sulforaphane was as indirect antioxidant . Keck and Finley [4] and Ding *et.al.* [5] also said that sulforaphane was produced through hydrolisis of glucosinolat. Then Berhow *et.al*. [6] expressed that one of

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glucisinolat group was glucoraphanin which due to the support of mycrocine forming sulfroraphane. According to Zhu *et.al.* [7], broccoli seed was rich of mycrosynase and glucoraphanin. Then, Chuanphongpanich *et.al*. [8] presented that level total of glucosinolat for the type of glucoraphanin at the seed of 5 variates like GreenKing, Packman, PaGing, Rod Fai, and TopGreen#67 was in the range of 11.4 until 48 umol/g DW, it was higher than the other type of glucosinolate. Farnham *et. al*. [9] expressed that result of glucoraphanine at some of genotype inbred like USVL102 of opened pollination reached 91 umol/g of seed, USVL049 of dihaploid reached 80,5 g/seed, but Pinacle of F1 reached 107,5 umol/g of broccoli seed. So they concluded that genotype determined the content of glucoraphanine. Content of glucoraphanine at this seed was different with the result of flower which researched by Sarikamis *et al* [10] that was at retail type of 1, 2, 3, Heritage,Marathon and (48-13-4 x Br9). Results of glucoraphanine (methylsulfinilbutyl) indicated that the highest was at the type of (48-13-4 x Br9) that was 10,6 umol/g

. According to sarikamis *et al* [10], initial precursor in synthesis of sulforaphane at vegetation of *Cruciferaceae* was metionine. But the enf of precursor fitted tp the line of sulforaphane compund synthesis was glucoraphanine. Research of Caridi *et.al.* [11] indicated that broccoli seed had the content of glucoraphanine between 20 until 50 mg/g of seed. But based on the research of Nagawa *et al* [12], it presented that content of sulforaphane at broccoli seed was 1153 mg / 100 g of dry weight and it was 10 times higher than grown up vegetation that was 44 until 171 mg/100 g of dry weight. Glucoraphanine in broccoli was as precursor which forming sulforaphane as phytochemical compound which was useful for healthy [13]. High content of glucoraphanine at broccoli seed could produce extract as the substitution of glucoraphanin for accelerating the synthesis of sulforaphane. Therefore, increasing sulforaphane could be carried out either with essence of growth regulator it could also add precursor of metionin and extract of broccoli seed at growth media so that could increase synthesis of sulforaphane.

This Study was referred to the researchs of many kinds of in vitro plant. Research of influence of sitokinin of BAP type on accumulation steroidal saponin at Solamun aviculare about the increasing secondary metablism compound indicated that result of saponin was bigger about 8 mgl at the culture of cell agregate which was given 10^{-8} MBAP [14]. Beside that, there was the increasing at *Dioscorea deltoidea* through callus culture of the plant. It was said that increasing 100 ppm pf cholesterol to callus culture media of *Dioscorea deltoidea* would increase the product of diosgenin until 100 % [15] Participation of amino acid in increasing secondary metablism compound had benn reported in biosynthesis of hyperforin and adhyperforin at the sproud culture of *H. Perforatum* that was by using Valin and Isoleusin sp taht adhyperforin was increasing 3 until 7 times [16]. Beside that, the usage of percursor in producing katarantin tapak dara could increase at the treatment of tryptophan for 100 – 225 ppm which was added 2 ppm and kinetin of 0,2 ppm at cell culture of C. roseus [17].

MATERIALS AND METHODS

1. Culture of network

This part of research was conducted at Laboratory of Biotechnology, Faculty of Agriculture, University of Sam Ratulangi during 4 months. Explant used in this research was sproud of induction at initiation level. Perfect random design was used in this research which factorially arranged as follow:

Factor of A: metionine and factor of B: extract of broccoli seed with each dosis
Factor of A: Metionine of 0; 50; 100: 150 ppm (M0; M50, M100; M150)
Factor of B: extract of broccoli seed of 0; 1; 2; 3 g (E0, E1, E2, E3)

Each treatment was included 4 returns. There were 3 times returns for each treatment of sulforaphane analysis. Media used in this research was MS and it was added 1 ppm of NAA and 5 ppm of BAP for increasing the sprout. Observed variables included number of sprout, wet weight of sprout, number of leaves, height of sprout, and content of sulforaphane. Data was observed at fourth week after culture. Then it was carried out analysis of variance and BNT test of 5%.

2. Analysis of Sulforaphane

This part of research was conducted at Laboratory of Biotechnology, Faculty of Engineering, University of Sam Ratulangi, Menado, Sulawesi of Indonesia and Laboratory of Chemical Engineering, Public Polythecnic, Malang of Indonesia. It was carried out during 1 year.

Utilization and material of research

Some utilizations were used in this research those were flow caPublic binet, freezer, oven, culture room, stirrer, rotary evaporator, pH meter, anaylitical balancer, detector, sonicleaner, hot plat, centrifugate,

mortal, and the other glasses, Liquid Chromatography Mass spectroscopy Mass Spectroscopy, Merk Accela 1250,Thermo Scientific, and detector type was MSMS type triple quadrupole merk TSQ quantum Acces Max of Thermo Scientific. Colom was used Hypersil gold with specification 50mm x 2.1 um. . Materials used in this research included media component of MS, NAA, BAP, jely, sucrose, and sprouts of induction and chemical compound for extraction, methanol p.a, acetonitril, ammonium format and aquadest, standard sulforaphane, and extract of broccoli seed. Variables in this study were qualitatively and quantitatively observed.

Extraction and isolation of sulforaphane

Extraction of sulforaphane was begun with measuring the weight of broccoli sprouts using digital measurer. Then the sprouts were entered at mortal and adding 1-2 ml of methyl chloride and smoothing them. The smooth broccoli sprout was moved into flash tube and adding it with 25-50 ml of methyl chloride. After that there was carried out sonofication during 30 minutes to bring out sulforaphane from broccoli sprout network. Extract product of sulforaphane was filtered using waltmen paper and being moved to a tube and putting it at heater block or hot plate with temperature of 70^0 to 80^0 C until producting supernatant.

The dry residu was added with 5 ml of $NaSO_4$ and was re-heated at hot plate with the temperature of 70^0 to 80^0 C until the supernatant was dry. Then, this residu was added with 10 ml of asetonitril, after that it was filtered with waltmen paper and the solution was sentrifugated during 15 minutes by 4000 rpm. At the end, the solution with residu content was moved at microtube and entered to LC tandem MS for determining content of sulforaphane sprout.

Qualitative and quantitative analysis of sulforaphane

Qualitative and quantitative analysis of sulforaphane was carried out using the utilization of LC MSMS Plus. Movement phase of A: 5 mmol/l of format-water, movement phase of B: asetonitril, flowe rate of 0.3 ml/minutes, injection volume of 5 ul, colum temperature of 40^0 C, gas flowing of 1.5 l/minutes, dry gas pressure of 10 l/minutes. Before carrying out the analysis, it was filtered to movement phase and solution of sample with membrane filter of selusose asetat (PTFE) of 0.4 um. Standard sulforaphane was used as comparison compound and it was got from the center of Scientific Biofarma. Qualitative analyses was intended to compare retention time between standard sulforaphane and sample. If there was compound with retention time was the same as standard sulforaphane, this compound was sulforaphane. Standard and sample co-injection was carried out to have a certainty that there was sulforaphane at sample.

Quantitative analysis was carried out to get concentration of sulforaphane. It was produced by conversing number area of sample with standard number area which had been known its concentration at standard calibration curve. Standard calibration curve was got from number area data on some standard concentration of sulforaphane and then there was made a relation between number area and the content of sulforaphane.

Analysis of data

After doing the collection of data, data was tabulated and then it was statistically analyzed for producing the conclusion. The content of sulforaphane at shoot, and Analysis data was using analysis of variance on factorially Perfect Random Design. If there was difference, it was continued with 5% of BNT test.

RESULTS AND DISCUSSION

Number of shoot

Result of variance analysis indicated that there was not intercation between metionine and extract of broccoli seed in the influence due to the number of shoot. In meant that the two treatments was not inter supporting to the number of shoot. Treatment influence of broccoli seed extract was not significant difference to the number of shoot. The result of total and averaged of shoott number on these treatments was high enough, but the results were almost the same, so that was not significant different. It indicated that concentration of 0 to 3 g broccoli seed extract was still the same in the influence to sprount number, but inthe higher concentration there was different. Vegetable of *Brassica Oleracea Var italica* had content of fatty, protein, carbohydrate, fibre, water, iron essence, calcium, mineral, and kind of vitamines like A, C, E, riboflamin, nikotinamide [18]. There was content of some compounds like this in broccoli seed and it was very important for the growth of shoots being grew in culture. Result of metionine treatment indicated that

there was significant different in the influence of sprout number. The highest sprout number was at the treatment of 50 mg of metionine. It indicated that sprout number at the treatment of 100 mg and 150 mg, trend of sprout forming was down and down when concentration of metionine was increasing. It might be caused by concentration was so high so that could pursue sprout forming (Table 1).

BNT test of 5% indicated that treatment on 50 mg of metionine was significant different with the treatment on 100 mg and 150 mg of metionine, but there was not significant different without metionine (or 0 mg of metionine) in the influence to sprout number. Treatment on 100 mg and 150 mg of metionine was not significant different to sprout number. Pursueing in sprout forming was occured at treatment on 100 mg and 150 mg of metionine to sprout forming. There was trend of little different at the two treatments as described in Table 1 and it indicated that higher concentration of metionine was not influenced sprout number. The two concentrations were so high so that was not good for supporting in sprout forming.There were a lot of glysine at the concentration of amino acid which was given at media of MS, that was 2 mg/l [19]. Mentionine might also be not too high in supporting of sdventive sprout forming, but it was more suitable as precursor function due to in vitro synthesis of sulforaphane from broccoli sprouts.

. Table 1 The influence of metionine to the number of sprout

Treatment	Number of sprout in average
M 0	12,81 b
M 50	15,75 b
M 100	3,50 a
M 150	2,25 a
BNT 5%	3,32

Fresh weight of shoot

Result of variance analysis indicated that there was interaction between metionine and extract of broccoli seed in the influence to wet weight of sprout. Both of the essences were inter supporting in the influence to wet weight of in vitro broccoli sprout. The highest wet weight of sprout at extract treatment without metionine was 3 g/l. But the highest of giving seed extract was 3 g/l, it was combined with metionine of 50 mg/l and 150 mg/l at media and it very pursued sprout forming.

BNT test of 5% indicated that treatment without metionine (0 mg of metinonine) and 0 g of broccoli seed extract was not have significant difference compared to treatment without metionine which was combined with 1 g and 2 g of broccoli seed extract in the influence to wet weight of shoot. The three treatments had the same influence, it meant that there was the same function until at the concentration on 2 g of broccoli seed extract. So concentration of seed extract was still very low and it meant there was no different function among one to others. At concentration on 2 g of seed extract there was trend on wet weight increasing, as presented in Table 2. But if the three treatments were compared with the combination on 0 mg of metionine (without metionine) with concentration on 3 gr of broccoli seed extract, it indicated that there was significant different in the influence to wet weight of adventive sprout. Therefore, the giving 3 g of broccoli seed extract to MS media was very good influence for wet weight of adventive sprout, as described in Table 2.

BNT test of 5% indicated that the treatment on 50 mg of metionine without broccoli seed extract (o g of seed extract) had significant difference on wet weight of adventive sprout if it was compared with combination on 50 mg of metionine with 1 g, 2 g, and 3 g of broccoli seed extract. But combination between 50 mg of metionine with 1 g, 2 g, and 3 g of broccoli seed extract was not significant different in the influence to wet weight of sprout. It might be not functioned again as supporting in sprout growing. In the other hand, it pursued sprout growing so that decreased wet weight of sprout. Therefore it was enough only at metionine of 50 mg/l was added at media and if it was combined with broccoli seed extract there would pursue in wet weight of adventive sprout. Metionine of 50 mg was very good for supporting wet weight of sprout.

The treatment on 100 mg and 150 mg of metionine which was combined with 0 g, 1 g, 2 g, and 3 g of broccoli seed extract indicated that there was not significant different in the influence to wet weight of sprout. It might be caused by concentration of both essences which was given in culture nedia was so high, so that the growing was so much pursued and wet weight of sprout was also very low at each combination.

Interaction result of combination on 1 g of broccoli seed extract and 0 mg, 50 mg, 100 mg, and 150 mg of metionine indicated that there was not significant different in the influence to wet weight of sprout. At the end, interaction result indicated that the treatment on 2 g and 3 g of broccoli seed extract which was combined with 0 mg, 50 mg, 100 mg, and 150 mg of metionine had significant difference in the influence to wet weight of sprout. BNT test of 5% which the treatment on 2 g and 3 g of broccoli seed extract without metionine (0 g of metionine) was significant different with the treatment on 2 g and 3 g of broccoli seed

extract which was combined with 50 mg, 100 mg, and 150 mg of metionine in the influence to fresh weight of sprout. But sprout weight was not significant different if the treatment on 2 g and 3 g of seed was combined with 50 mg, 100 mg, and 150 mg of metionine. It might be the concentration had been so high, so that sprout weight was rather pursued, as presented in Table 2.

Table 2 The influence on intercation of metionine and broccoli seed extract to wet weight of sprout

Treatment		**Broccoli seed extract/g / Wet weight of sprout in average**			
		0	1	2	3
Metionine/mg	0	0,508 [b]	0,398 [ab]	0,746 [bc]	1,179 [d]
	50	0,928 [cd]	0,303 [a]	0,177 [a]	0,09 [a]
	100	0,171 [a]	0,245 [a]	0,174 [a]	0,110 [a]
	150	0,194 [a]	0,131 [a]	0,123 [a]	0,054 [a]
	BNT 5 %		0,39		

Height of shoot

BNT test of 5% indicated that treatment on 0 mg of metionine (without metionine) was significant different for sprout height in average with treatment on 50 mg/l, 100 mg/l, and 150 mg/l. Then shoott height in average on the treatment of 50 mg was not significant different with treatment on 100 mg but it was significant different with treatment on 150 mg of metionine. But sprout height in average on treatment of 100 mg was not significant different with treatment on 150 mg of metionine. Shoot height in average on the treatment without metionine was higher than the treatment on 50 mg/l, 100 mg/l, and 150 mg/l of metionine which was given to media. So much high the concentration of metionine which was given to MS media would cause so much low the sprouts at this media. It meant that high concentration would pursue height increasing og broccoli sprout at this media, as presented in Table 3.

The giving of seed extract at MS media with different concentration caused the influence was significant different due to the height of sprout. Shoot height in average on the treatment of 0 g or without seed extract was significant different compared with the treatment on seed extract which was given at the media of 1 g, 2 g, and 3 g. This result indicated that without giving of seed extract, it had been enough for the increasing of broccoli sprout height because with addition of broccoli seed extract, it could pursue the increasing of in vitro broccoli shoot height. Treatment on 1 g, 2g, and 3 g of seed extract was not significant different in the influence to shoot height, as presented in Table 4. It might be at the extract there was compound content which had characteristic of pursueing the growth so that the sprouts grew slowly. Slow growth could produce not different of sprout height. It could showed that so much high the concentration of sprout height in average caused so much short the shoot.

Table 3 The influence of metionine to shoot height

Treatment	**Sprout height in average (cm)**
M 0	3,06 c
M 50	2,01 b
M 100	1,67 ab
M 150	1,37 a
BNT 5%	0,47

Table 4 The influence of broccoli seed extract to sprout height

Treatment	**Sprout height in avearge (cm)**
E 0	2,53 b
E 1	2,04 a
E 2	1,92 a
E 3	1,62 a
BNT 5%	0,47

Number of leaf

BNT test of 5% indicated that there was not significant different of leaf number on the treatment only for extract of broccoli seed without metionine. It might be caused by the giving concentration until at 3 g of extract could support the influence to leaf number. Then, combination between 50 mg of metionine with 0 g, 1 g, 2 g, and 3 g of broccoli seed extract indicated significant difference in the influence to leaf number. But

only at combination between 50 mg of metionine and 0 g of seed extract had significant difference with 1 g. 2 g, and 3 g. But number of leaf was not significant different at the combination on 50 mg of metionine with 1 g of broccoli seed extract compared to leaf number at the combination on 50 mg of metionine with 2 g and 3 g of broccoli seed extract. It might be caused by the two essences would inter pursue after being given together so that the increasing of leaf number was also pursued. In further reason, addition of seed extract which was combined with metionine, had caused more material of food for the sproud so that pursued the number of leaf on sproud performing. Then, treatment on 100 mg and 150 mg of metionine which was combined with 0 g, 1 g, 2 g, and 3 g of broccoli seed extract indicated that number of leaf was not significant different and the number was so much little. It meant that there was pursueing in the influence to the number of leaf, as presented in Table 5.

Analysis of variance indicated that broccoli seed extract due to metionine had significant difference in the influence to the number of leaf. After being carried out 5% of BNT test, it indicated that number leaf at combination on 0 g of seed extract and 0 g of metionine was not significant different with combination on 0 g of seed extract and 50 mg of metionine, but there was different with combination on 0 g of seed extract and 150 mg of metionine. Number of leaf at combination on 0 g of seed extract with 100 mg was not significant different compared with combination on 0 g of seed extract with 150 mg. It was caused that there was high concentration of metionine which would pursue leaf forming. Combination treatment on 1 g of seed extract with 0 mg of metionine for leaf number was not significant different with combination treatment on 1 g of seed extract with 50 mg of metionine but it was significant different on leaf number compared with combination treatment on 1 g of seed extract with 100 mg and 150 mg of metionine.

At the end, treatment on 2 g and 3 g of broccoli extract seed without metionine was significant different on leaf number and it was more than treatment on 2 g and 3 g of seed extract which was combined with 50 mg, 100 mg, and 150 mg of metionine. Then, leaf number was not significant different at the treatment on 2 g and 3 g of seed extract which was combined with 50 mg, 100 mg, and 150 mg of metionine, as presented in Table 5.

Based on the result as above, it indicated that so much high the concentration of treatment on broccoli seed extract which was combined with metionine would cause pursueing to the leaf number of adventive sp

Table 5 The influence on interaction of metionine and broccoli seed extract to the number of leaf

Treatment		**Extract of broccoli seed/g / Number of leaf in average**			
		0	1	2	3
Metionin/mg	0	18,25 ^{c}	15,75 ^{bc}	22,00 ^{c}	20,75 ^{c}
	50	23.50 ^{c}	8,75 ^{ab}	7,00 ^{a}	5,50 ^{a}
	100	4,00 ^{a}	7,50 ^{a}	3,50 ^{a}	5,75 ^{a}
	150	4,00 ^{a}	3,75 ^{a}	6,50 ^{a}	2,50 ^{a}
BNT of 5 %				8,21	

Sulforaphane content

BNT test of 5% indicated that treatment of sulforaphane on 0 mg of metionine which was combined with 0 g of seed extract was not significant different with combination treatment on o mg of metionine with 1 g and 2 g of broccoli seed extract but it was significant different with combination treatment on o g of metionine and 3 g of broccoli seed extract. Sulforaphane content at the treatment on 50 mg of metionine which was combined with 0 g of broccoli seed extract was significant different with combination treatment on 50 mg of metionine and 1 g, 2 g, and 3 g of broccoli seed extract. But sulforaphane content at combination on 50 g of metionine and 1 g of seed extract was not significant different with combination treatment on 50 mg of metionine and 2 g and 3 g of broccoli seed extract. Sulforaphane content at the treatment on 100 mg/l which was combined with 0 g of seed extract was significant different with combination treatment on 100 mg of metionine and 1 g, 2 g, and 3 g of broccoli seed extract, even the four combinations were significant different in sulforaphane content which was synthesized from boccoli seed. Combination on 150 mg of metionine and 0 g of seed extract was significant different with combination on 150 mg of metionine and 1 g, 2 g, and 3 g of broccoli seed extract. But sulforaphane content of adventive sproud at combination trewatment on 150 mg of metionine with 1 g of seed extract was not significant different with combination on 150 mg of metionine and 2 g of broccoli seed extract.

Combination on 100 g of metionine with 1 g of seed extract was the highest sulforaphane content of sprout, that was 182.09 ng/g of plant material (sprouds). This was the best combination for sulfaraphane synthesis on adventive sprouts. But for single giving was at the concentration on 100 mg of metionine that was 162.89 ng/g of plant material (adventive sprouds). There was still very little for seed extract compared to metionine as well as combination of metionine and broccoli seed extract which was given at media of sprout

increasing. But, treatment on 1 g of seed extract which was given to media of sprout increasing, produced sulforaphane content higher than with treatment on 0 g, 2 g, and 3 g of broccoli seed extract that was with sulforaphane content of 32.59 ng/g of plant material (sprout), as presented in Table 6.

Based on number, wet weight, and height of sprout, and number of leaf, it indicated that so much high the concentration of metionine as well as broccoli seed extract caused so much short its size. On the other hand, at sulforaphane content of sprout, high concentration of metionine even it was combined with seed extract, would produce high sulforaphane content. Therefore, so much be pressured the growth, sulforaphane content was so much high, as presented in Table 6. The result of sulforaphane content was still much less than field result.

Table 6 The influence of interaction between metionine and extract of broccoli seed due to the content of sulforaphane

Treatment		**Extract of broccoli seed/ g/ averaged sulforaphane content (ng)/ g wet weight of sprout**			
		0	1	2	3
Metionine/mg	0	26,05 ^{ab}	32,59 ^{bc}	23,39 ^{ab}	9,58 ^{a}
	50	12,68 ^{a}	45,22 ^{cd}	44,68^{cd}	54,92^{def}
	100	162,89 ^{i}	182,09 ^{j}	47,48^{cde}	83,07 ^{g}
	150	36,20 ^{bc}	63,14^{ef}	69,37^{fg}	139,32 ^{h}
	BNT 5 %			17,45	

CONCLUSION

Based on the observation and analysis as above, it was concluded as follow. There was intercation between metionine and extract of broccoli seed in the influence due to the fresh wieght of .sprout. Concentration on 3 g of broccoli seed extract which was given to media of MS was very good due to fresh weight of sprout. The best influence of concentration on 50 mg of metionine was for fresh weight of sprout. The best combination for the content of sulforaphane was at the combination treatment on 100 mg metionine and 1 g extract of broccoli seed.

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