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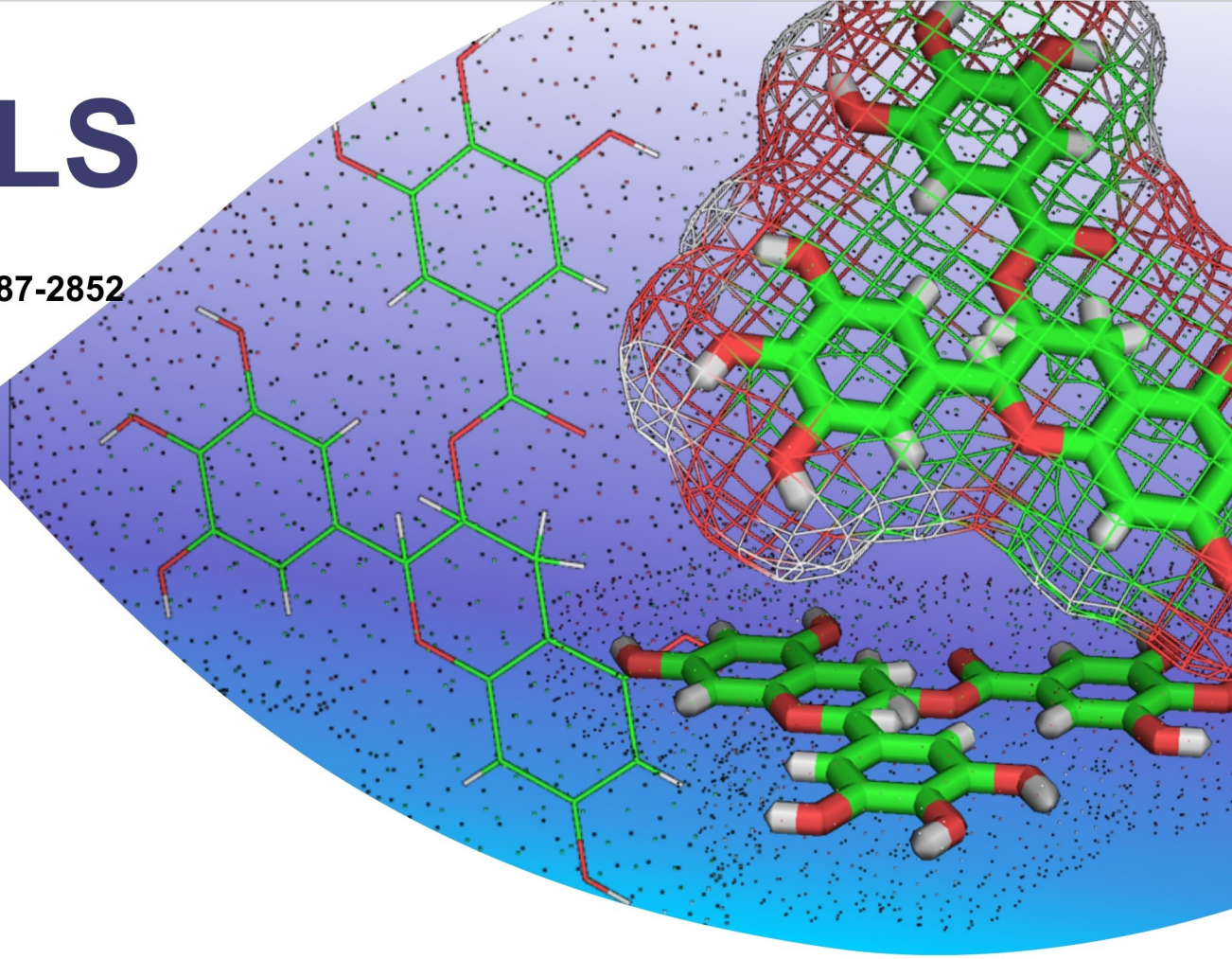

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Genetic Variation Analysis of Four Local Varieties of Indonesian Black Rice (*Oryza sativa* L.) Based on Partially *rbc*L cpDNA Gene Sequence

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Abstract

Black rice (*Oryza sativa* L.) varieties i.e. Toraja (South Sulawesi), Cempo Ireng (Yogyakarta), Wojalaka (East Nusa Tenggara), and Manggarai (East Nusa Tenggara) are four local black rice varieties in Indonesia whose character has not been widely studied, especially the character of genetic variation. Research aimed to determine the variation of the *rbc*L gene in the four local black rice varieties. The sample for testing the variation of the *rbc*L gene sequence in the form of black rice leaves six weeks after planting. Dendogram was carried out using the UPGMA method with the Kimura 2-parameter algorithmic calculation model using the MEGA5 version 5.2.2 program. The results showed that partially the *rbc*L gene sequence was successfully amplified on four black rice varieties with a sequence length of 487 bp. The partial *rbc*L sequence of black rice consisted of 26.58% tyrosine, 21.38% cytosine, 28.86% adenine, and 23.18% guanine. The value of G + C content was 0.446, with the frequency of invariable sites of 97.13%. The frequency of informative parsimony sites was 1.43% with a nucleotide diversity (Pi) value of 42-10, the number of haplotypes was 5, and the total number of mutations and polymorphic sites was 14. The ratio between transition and transversion (ts/tv ratio k) for purine bases was 1.741 and pyrimidine was 3.571, with the estimated overall ratio between transition and transversion (R) of 1.31. Based on the dendogram, the farthest genetic distance was found in Wojalaka and Manggarai varieties, which were 0.019 respectively.

Keywords: black rice, genetic variation, local varieties, *rbc*L gen

INTRODUCTION

Black rice (*Oryza sativa* L.) is one type of rice in the world. In addition to white rice, brown rice, and brown rice [1], black rice has become popular and is consumed by some people as a functional food. Petroni and Tenolli [2] suggest that the increase in demand for functional foods is due to the high content of antioxidants, such as anthocyanin pigments. Scientific studies on Indonesian local black rice are few, and information about black rice, which has more potential as a functional food, is also limited.

The *Toraja* variety (Sulawesi), the *Cempo Ireng* variety (Yogyakarta), the *Wojalaka*, and the *Manggarai* varieties (East Nusa Tenggara) are four local black rice varieties in Indonesia that have not been widely studied, particularly concerning genetic variations and anthocyanin levels. Complete information can support the efforts of breeding and conservation of the four black rice varieties.

Genetic variation within a species is often influenced by the reproductive behavior of individuals in that population. Genetic variation arises because each individual has unique gene forms. Genetic variation occurs through mutation and recombination mechanisms [3]. One of the approaches used to study genetic variation is the DNA barcode. DNA barcode is a sequence or sequence of nucleotide bases of DNA or certain short genes, taken from one or more standardized genomes, used for the fast and practical identification and discovery of species [4]. Most of the genes that were used as DNA barcode in plants are those contained in the chloroplast DNA (cpDNA) genome. The selection of the barcode gene on cpDNA is due to the higher number of nucleotide substitutions compared to the genomic mitochondrial DNA (mtDNA). Besides that, cpDNA in most plant species is uniparentally derived, making it easier for evolutionary studies [5].

The barcode gene used in this study is the rbcL gene contained in cpDNA. The rbcL gene is a gene that encodes the key protein ribulose-1,5-biphosphate carboxylase-oxygenase (abbreviated as RuBisCO), which participates in carbon fixation in the photosynthesis process [6]. The rbcL gene is about 1400 bp (base pairs) in length, so it provides many characters for phylogenetic studies [7]. Cumming *et al.* [8] explained that the database on the *rbc*L gene was owned by many species, making it easier to compare in data analysis. Based on a database that can be accessed through the official NCBI (National Center for Biotechnology Information) website, to date, more than 143 partial nucleotide sequences of the *rbc*L gene from the genus *Oryza* have been collected. The purpose of this study was to reveal the genetic variation in the four

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local black rice varieties based on partial *rbc*L gene sequences.

MATERIAL AND METHOD

Samples and DNA Extraction

The samples of the seeds of four black rice varieties used in this study were obtained from local farmers in each region. Partial analysis of *rbc*L gene sequences was carried out on extracted DNA from black rice leaf tissues six weeks after planting.

The extraction of black rice genomic DNA in this study used the Wizard® Genomic DNA Purification Kit (Promega Corporation) following standard protocols. Measurement of the concentration and purity of isolated DNA was carried out qualitatively and quantitatively. Qualitative measurements used 1% agarose gel electrophoresis with a 1 kb DNA ladder comparison (VC 1kb DNA ladder) using a Nanodrop2000 UV spectrophotometer machine. Quantitative measurements of DNA purity and concentration were carried out by taking 2 µl of DNA samples then added with 995 µl of Tris-EDTA (TE) buffer and placed in the vortex machine until the solution was homogeneous. Then the solution was put in a cuvette and its absorbance was measured at a wavelength of 230 nm, 260 nm, and 280 nm.

Amplification of partially *rbc*L gene sequences.

The primers used in this study were universal primers of the rbcL gene. The forward primer used was rbcLa-F with the 5'-ATGTCACCACAA ACAGAAAC-3 'arrangement developed by Kress and Erickson [9] and the reverse primer used was rbcL-724R by *Fay et al.* [10] with the 5'-TCGCATGTACCTGCAGTAGC-3'. The PCR cycles performed were predenaturation at 95°C for 5 minutes, denaturation at 95°C for 45 seconds, annealing at 60.8°C for 45 seconds, extension at 72°C for 45 seconds, and post extension at 72°C for 10 minutes. The main cycle (denaturation, annealing, and extension) of amplification was repeated 35 times. Confirmation of isolated DNA and *rbc*L gene amplicon was carried out using 2% agarose gel electrophoresis.

Sequencing of partially rbcL gene sequences.

Purification and sequencing were carried out by 1st base (Selangor, Malaysia) through distributor PT. Genetics Science Indonesia. Sequencing was performed using the ABI PRISM 3730xl Genetic Analyzer (Biosystem, USA). The standard protocol used was the BigDye® Terminator v3.1 Cycle Sequencing Kit. The sequencing results read by BioEdit program.

Sequence Alignment and Data Analysis.

The validity test of DNA sequences was carried out using the BLAST program, which can be accessed online on the NCBI website. Meanwhile, the partial reliability test of the rbcL gene sequences was carried out using the BioEdit program. Next, sequence alignment was carried out to determine the homology of a DNA sequence with other DNA sequences. The sequence alignment used in this study was multiple alignments because it involves many partial homologous gene sequences. Multiple alignments were done using ClustalW. Dendogram tree construction used the UPGMA (Unweighted Pair Group Method with Arithmetic Means) method with the algorithmic calculation of the 2-parameter Kimura substitution model. A total of 12 accessions to the partial comparison of *rbc*L gene sequences were selected from Genbank. The list of accessions for comparisons was described in Table 1. Evaluation of the phenogram tree was carried out using a bootstrap test with 1000 replications. Multiple alignments and construction of phenogram trees were carried out using the MEGA5 (Molecular Evolutionary Genetic Analysis) program 5.2.2.

Table 1. List of 12 accessions used for comparison

No.	Accession codes	Species description	Code for analysis
1.	KF731215.1	Oryza *sativa* Japonica Group bio-material RGCB#CR145	Os Japonica Group 1
2.	KF731214.1	Oryza *sativa* Japonica Group bio-material RGCB#CR383	Os Japonica Group 2
3.	KF731204.1	Oryza *sativa* Japonica Group bio-material IRGC:9147	Os Japonica Group 3
4.	KF731201.1	Oryza *sativa* Japonica Group bio-material RGCB#CR380	Os Japonica Group 4
5.	KF731220.1	Oryza *sativa* Indica Group bio-material RGCB#CR487	Os Indica Group 1
6.	KF731218.1	Oryza *sativa* Indica Group bio-material RGCB#CR495	Os Indica Group 2
7.	KF731217.1	Oryza *sativa* Indica Group bio-material RGCB#CR666	Os Indica Group 3
8.	KF731212.1	Oryza *sativa* Indica Group bio-material RGCB#CR40	Os Indica Group 4
9.	KF731190.1	Oryza *rufipogon* bio-material RGCB#Kor138	Oryza rufipogon 1
10.	KF731189.1	Oryza *rufipogon* bio-material RGCB#Kor124	Oryza rufipogon 2
11.	JQ593378.1	Oryza *latifolia* voucher BioBot01092	Oryza latifolia 1
12.	JQ593377.1	Oryza *latifolia* voucher BioBot01091	Oryza latifolia 2

(**Source**: NCBI, 2014)

RESULT AND DISCUSSION

Partial Extraction and Amplification of the *rbc*L gene sequence

Partial amplification of rbcL gene sequences with universal primers was successfully carried out with rbcLa-F forward primers and rbcL-724R reverse primers. The results of electrophoresis on 2% agarose gel showed that the partial length of the amplified rbcL gene sequence was 600 bp (Fig. 1).

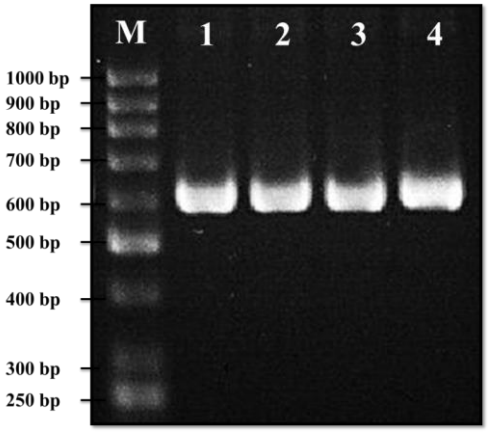

Figure 1. Visualization of 1% agarose gel electrophoresis as the result of genomic DNA isolation of four black rice varieties; M: Marker 1 kb, 1: Toraja variety, 2: Cempo Ireng variety, 3: Manggarai variety, and 4: Wojalaka variety

Sequence's characteristics.

Partial characteristics of the 487 bp rbcL gene sequence obtained from black rice varieties Cempo Ireng, Toraja, Wojalaka, and Manggarai were compared to 12 accessions of Genus *Oryza* obtained from Genbank. It showed the characteristics of nucleotides composed of 26.58% tyrosine, 21.38% cytosine, 28.86% adenine, and 23.18% guanine (Table 2). The value of G + C content is 0.446, with the frequency of invariable sites of 97.13%. The frequency of informative parsimony sites is 1.43% with a nucleotide diversity (Pi) value of 42-10, the number of haplotypes is 5, and the total number of mutations and polymorphic sites is 14. The ratio between transition and transversion (ts/tv ratio k) for purine bases is 1.741, and pyrimidine is 3.571, with the estimated overall ratio of transition and transversion (ts/tv R) of 1.31.

Partial comparison of rbcL gene sequences of black rice with 12 accessions from GenBank showed 473 monomorphic sites (invariable) and 14 polymorphic sites (variable). These 14 polymorphic sites show that 14 nucleotide base points have mutations. Singletone variations are found in the positions of the nucleotide base sequences 131, 174, 206, 212, 216, 220, and 419, while the informative parsimony variations are in the positions of the nucleotide base sequences 35, 128, 154, 162, 296, 424, and 440. According to Yingzhi *et al.* [11], the single tone variation is a site where only one taxon is different. Meanwhile, the informative parsimony site is an informative site with a minimum characteristic of being composed of two nucleotides, and both must appear at least twice on the site.

Singletone variations occur because of mutations, both transitional mutations, and transversion mutations. According to Pierce [6], a transition mutation is the replacement of a purine nucleotide base with a purine or pyrimidine nucleotide base with a pyrimidine, while a transversion mutation is the replacement of a purine nucleotide base with a pyrimidine or vice versa. In the Manggarai variety, variations at positions 174, 206, 212, and 220 were caused by a transfer mutation, while 131 and 419 were due to a transitional mutation.

Table 2. Characteristics of partially *rbc*L gene sequences.

Parameter	Results	Total
Frequency of tyrosine (T)	26.58%	487
Frequency of cytosine (C)	21.38%	487
Frequency of adenine (A)	28.86%	487
Frequency of guanin (G)	23.18%	487
G + C *content*	0.446	
Invariable site frequency	97.13%	
Frequency of informative parsimony sites	1.43%	
Diversity of nucleotide (Pi)	0.0000042	
Number of haplotypes	5	
Total number of mutations	14	
Polymorphic site	14	
Estimated overall ts/tv ratio (k)	Purine = 1.741 Pyrimidine = 3.571	
Estimated overall ts/tv ratio (R)	1.31	

Note: frequency analysis of nucleotide bases based solely on 487 bp

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Variations of informative parsimony at positions 35 and 440 occurred transitional mutations, while at positions 154 and 296 there were transversion mutations in comparison accessions of *Oryza latifolia* 1 and 2. At position 128 there were transitional mutations in Manggarai and Cempo Ireng, position 162 there were transition mutations in Toraja varieties and Manggarai, position 424, there are transitional mutations in the Toraja and Cempo Ireng varieties. The total number of mutations for the parsimony informative variation of the Manggarai variety is 2 and the overall number of single nucleotide variations is 7, this is thought to be the cause of the position of the Manggarai variety being in the outermost branch of the dendogram.

Other molecular markers have also been used to describe genetic variation in local Indonesian black rice, namely microsatellites or SSR (short tandem repeat) [1]. Based on the use of microsatellite markers, it is known that black rice varieties are genetically different from white rice and that there are intraspecies variations regardless of geographical origin. In line with the results of this study, the use of partial markers for the rbcL gene sequence also showed that there were genetic variations in these four black rice varieties regardless of their geographic origin. This kind of genetic variation analysis still needs to be carried out as an initial step for the identification of black rice varieties and the selection of parent crosses in assisting black rice breeding programs in Indonesia.

Genetic Distance and Dendogram.

Dendogram construction was carried out using the UPGMA method with the Kimura 2-parameter algorithmic calculation model (Fig. 2). The UPGMA dendogram was constructed based on a 487 bp long sequence.

The farthest genetic distance was found in Wojalaka and Manggarai varieties, which were 0.019. This genetic distance showed that the kinship of the two varieties is quite far, even separated and isolated even though they are in one province. The Toraja and Cempo Ireng varieties were grouped into one node with a genetic distance of 0.04. There are 14 nucleotide base points that have mutations. Single nucleotide variations (single tone) are found at the positions of the nucleotide sequence 131, 174, 206, 212, 216, 220, and 419, while the informative parsimony sites are at positions of nucleotide base sequences 35, 128, 154, 162, 296, 424, and 440.

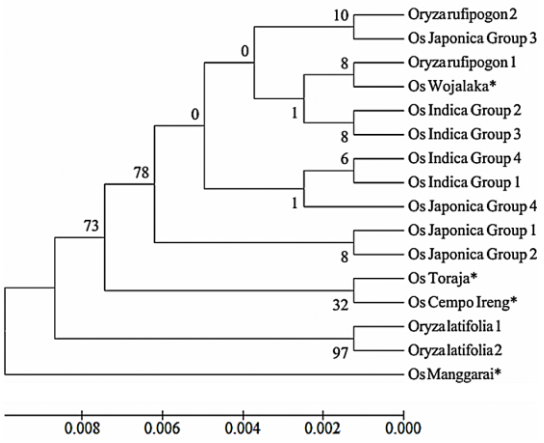

Figure 2. The dendogram with the UPGMA method (bootstrap 1000 replications) showed the genetic relationship of four black rice varieties; (*) samples of black rice varieties in this research.

CONCLUSION

The 487 bp long nucleotides tested in this study consisted of 26.58% tyrosine, 21.38% cytosine, 28.86% adenine, and 23.18% guanine. The value of G + C content was 0.446, with the frequency of invariable sites of 97.13%. The frequency of informative parsimony sites was 1.43% with a nucleotide diversity (Pi) value of 42-10, the number of haplotypes was 5, and the total number of mutations and polymorphic sites was 14. The ratio between transition and transversion (ts-tv ratio or k) for purine bases was 1.741 and pyrimidine was 3.571, with the estimated overall ratio between transition and transversion (R) of 1.31.

The UPGMA dendogram showed the farthest genetic distance was found in Wojalaka and Manggarai varieties. The Toraja and Cempo Ireng varieties were grouped into one node with a 0.04 genetic distance. There are 14 nucleotide base points that have mutations. Single nucleotide variations (single tone) found at the nucleotide sequence 131, 174, 206, 212, 216, 220, and 419, while the informative parsimony sites are at nucleotide base sequences 35, 128, 154, 162, 296, 424, and 440.

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The Sensitivity of *Leersia hexandra* Sw. to Gamma-Ray Irradiation

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Abstract

Gamma-ray irradiation as a physical mutagen has high penetrating power. Therefore, it is most often used to increase genetic variability or produce new mutant plants. This research was conducted to obtain the lethal dose of gamma-rays in *Leersia hexandra* plants. The used plant part was a single node stolon which had a length of 10 cm with the node in the middle of the stolon. The irradiation doses given were 0, 25, 50, 75, 100 Gy. Stolons were inserted into plastic clips and irradiated using a Cobalt-60 gamma irradiation source at the Center for Irradiation and Radioisotope Applications (PAIR), National Nuclear Energy Agency (BATAN) Jakarta. Gamma-irradiation has a significant effect on inhibiting shoot growth. The growth of irradiated *Leersia hexandra* with the best number of plants and the highest shoots was obtained at a dose of 25 Gy and decreased with increasing irradiation dose. The lethal dose (LD_{50}) was determined eight days after irradiation using CurveExpert 1.4 software. *Leersia hexandra* plants that can sprout and regenerate followed the linear equation y = 1.02 - 7.5x with LD_{50} at 68.85 Gy and LD_{20} at 29.36 Gy.

Keywords: gamma irradiation, genetic variation, *Leersia hexandra*, lethal dose, mutant plant.

INTRODUCTION

Forage is one of the determining factors in the development of the ruminant livestock business. The obstacle faced by breeders is the availability of a fairly good variety of forages in terms of quantity and nutritional quality. One plant that has been used as forage for livestock is the swamp plant *Leersia hexandra*. According to Riswandi [1], plants that grow in swampy areas have the potential to increase the feed diversity for livestock.

The natural swamp plants generally have limitations in their nutritional value, such as their protein content. Natural grass has a scarce protein content which is around 4% [2]. Due to overcome this limited nutritional value, a plant engineering effort is needed.

The engineering to obtain new varieties of plants can be done by mutation induction. In this method, there are two mutagens used, namely chemical mutagens and physical mutagens. Chemical mutagens include EMS (ethyl methanesulfonate), while physical mutagens include infrared and gamma rays.

Physical mutagens are influenced by the frequency and spectrum of irradiation as well as depending on the dose and dose rate used. Physical irradiation is very efficient in producing changes in genetic material [3]. Mutation by physical induction has become a technique for cultivar improvement. It is very effective in increasing natural genetic resources to obtain the desired mutant characteristics [4]. The unit of irradiation dose used in Gray (Gy), which is equivalent to the absorption of one Joule of radiation energy per kilogram of irradiated material [5]. Plants have a certain sensitivity value to irradiation, hereinafter referred to as Lethal Dose (LD). LD is measured at doses that cause death in plant populations [6].

A radiosensitivity assessment is carried out to determine the LD_{50}, which is the safe dose plants can survive to germinate in as much as 50% of the population. LD_{50} can be used as a basis for breeders to increase plant genotypes [7]. Aisyah [8] stated that the LD_{50} was different for each type of plant depending on the stage of growth, plant development, and the part of the plant that was irradiated. Instead of plant species, several factors might respond differently to gamma irradiation, including ploidy level, plant development stage, and physiological factors. Also, oxygen and water molecules (H_2O) contained in the material exposed to irradiation were directly proportional to the free radicals formed, which caused plants to be more sensitive to exposure to gamma irradiation [9].

Information on the optimum dose of irradiation is needed for researchers to produce mutant plants. Mutant plants obtained from gamma irradiation are generally at or slightly below the LD_{50} value [10]. Therefore, this study aims to determine the sensitivity of *Leersia hexandra* plants through analysis of LD_{50} values.

MATERIAL AND METHOD

The research was carried out in the experimental garden of the Beef Cattle Research Station. The gamma irradiation was conducted at

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the Irradiation and Radioisotope Application Center (PAIR), National Nuclear Energy Agency (BATAN), Pasar Minggu, Jakarta. The used plant material was *L. hexandra* stolon obtained from the experimental garden collection of the Beef Cattle Research Station. A stolon with one node was taken from a mature plant. It was chosen from the lower part near the roots of each stem. The stolon was 10 cm long, with the node in the middle of the stolon. The single-node-stolon pieces were stored in a plastic clip [11].

Gamma irradiation was conducted at doses of 0 (control), 25, 50, 75, 100 Gy using a gamma irradiation source Co-60, and temperature in the chamber 35-40°C. Each treatment dose consisted of 100 replications. The stolons as a control that were not irradiated by gamma rays remained in the plastic clip to keep the stolons moist until implanted. Immediately after irradiation, the stolons were implanted [11].

The used planting medium was soil, husk charcoal, and compost with a volume ratio (1: 1: 1). Stolons were planted in the planting medium in seedling polybags of 15x10 (cm) size then the medium was maintained to be moist. The air humidity also was maintained by providing a plastic cover after the stolons were planted in seedling polybag seedling; the used lighting was natural sunlight [12].

Observations were made on the sprouted stolons eight days after irradiation. The preliminary research results showed that the number of shoots growing from 100 stolons without irradiation was 69 shoots (control). Therefore, the percentage of the lethal dose was calculated based on the number of these living individuals. The lethal doses of 20 (LD_{20}) and 50 (LD_{50}) were determined by the CurveExpert 1.4 analysis program to find the best model equation. Further, plant shoot height was measured from the soil surface to the tip of the plant growth point. Plant height data were grouped into 4 clusters, including a height range of 0.5-4.5; 4.6-8.5; 8.6-12.5; 12.6-16.5. Individuals with height in each of these clusters were counted [13].

RESULT AND DISCUSSION

Shoot growth

L. hexandra plants reproduce vegetatively using stolons. Stolons are extensions of plant stems that spread horizontally above the soil surface and have a role as vegetative propagation organs for plants [14].

As explained in the materials and methods section, the preliminary study showed that *L. hexandra,* which did not obtain the irradiation dose, was able to grow as many as 69 individuals per 100 stolon samples. Therefore, the calculation of the percentage of shoot growth in subsequent treatments was based on this preliminary study. The percentage of shoot growth from *L. hexandra* stolons observed at the age of 8 days after irradiation obtained results as shown in Table 1.

Table 1. Percentage of Growing Shoots from Stolons

No	Irradiation Dose (Gy)	Percentage (%)
1	0	100*
2	25	84
3	50	62.5
4	75	50.7
5	100	21.7

Note: * 69 individuals living of 100 replicates

Based on table 1, the 25 Gy irradiation dose produced the survived stolons with a growth percentage of 84%. Similar to studies using the grass stolon Augustine genotype FA-243, Floratam, and the green mutant, at a dose of 3000 rads or 30 Gy irradiation, they produced the greatest number of lives [15].

At different irradiation doses, the response of survived stolons was different. Also, the higher the irradiation dose, the number of stolons that grown shoots decrease. The same results were shown by Zanzibar and Dede's research, that the highest irradiation dose of 100 Gy resulted in plants growing at the lowest as about 5.33% on *Magnolia champaca* [16]. It was due to the greater the dose of irradiation given, the more severe cell damage occurred, then plants could not survive and die [17]. Moreover, according to Al-Safadi *et al.*, the use of low-dose gamma-ray irradiation potentially stimulated plant growth in vivo [18].

The decrease in the number of plants that could sprout at higher mutagen doses might be due to cellular, physiological or physical disturbances. A study using Bermuda grass stolon showed that the higher the irradiation dose used, the percentage of shoot growth also decreased [11]. The same thing happened to chickpea seeds that received gamma irradiation, the higher the irradiation dose, the lower the percentage of germination [19].

Lethal dose

The success of mutations using gamma-ray irradiation depended on the irradiation dose used. The mutagenic effectiveness was decreased with the increase in the mutagen dose

used. It indicates that there was a negative relationship between both of them. The results of data analysis using CurveExpert 1.4 were presented in Figure 1. The LD_{50} value was obtained at a dose of 68.85 Gy while LD_{20} was at a dose of 29.36 Gy, with the linear equation y = 1.02-7.56x

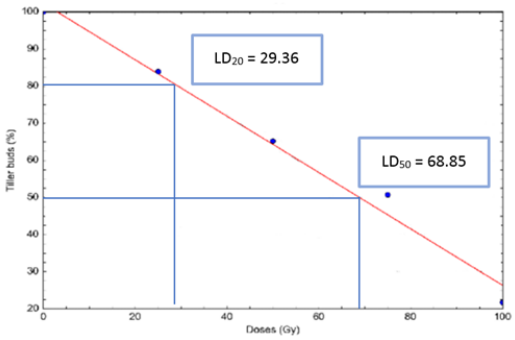

Figure 1. Determination of LD_{20} and LD_{50} in *L. hexandra*

The sensitivity of plants to gamma-ray irradiation varies, for example, the LD_{50} in Bermuda grass studies showed the irradiation that caused 50% plant mortality was at a dose of 85.45 Gy [11], whereas on Augustine grass ranged from 30 - 48.5 Gy [15], on *Anthurium andreanum* plants at dose 22.37 Gy [20], on *M. champaca plant* at dose 30 Gy [14]. It indicated that the LD_{50} yield is different for each species and also for varieties.

The level of plant sensitivity to gamma irradiation is affected by physical and biological factors. Physical factors, including plant morphology, may affect the physical resistance of cells in receiving gamma irradiation. Meanwhile, biological factors include genetic factors and environmental factors (oxygen, the water content of irradiated material, storage treatment after irradiation, and temperature) [17].

Plant height

Gamma-ray irradiation in small doses gave a positive response to plant height. The number of plants in each group/plant height range was presented in Table 2. The results of the 25 Gy irradiation produced plants that were higher than the control plants.

The irradiation dose of 100 Gy resulted in the shortest plant height, range 0.5 – 4.5 cm. The results of this study were the same as the research results shown by Zanzibar and Sudrajat in *M. champaca* plants at a low dose of irradiation, produced higher mutant plants compared to control plants, and at a dose of 100 Gy produced the shortest mutant plants [16]. Similar to Wahyuni's research, the 100 Gy dose on cassava plants produced the shortest mutants [21].

Table 2. Number of tiller bud growing from stolon under several irradiation doses on 10 DAI

Dose (Gy)	Number of plants*			
	0.5 - 4.5	4.6 - 8.5	8.6 - 12.5	12.6-16.5
0	41	13	2	1
25	20	14	2	2
50	31	7	0	0
75	14	4	0	0
100	8	0	0	0

Note: *the number of plants in the range of tiller bud height in centimeter units

The irradiated rice plants by gamma-rays also showed that the higher the irradiation dose, the lower the plant height [22]. The direct effect that arises as a result of giving gamma irradiation mutagens was the cell damage. Also, the decrease in plant height by the increasing dose of irradiation happened due to the damage or change of chromosomes. Further, it was expressed by mutant plants through changes in plant morphology, physiology, and biochemical content [23]. Disruption in DNA synthesis and physiological and biochemical changes after gamma-ray irradiation could cause a decrease in plant height. The variation of shoot growth of *L. hexandra* on 10 DAI was presented in Figure 2.

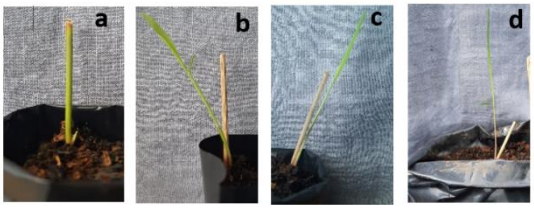

Figure 2. Variation of shoot growth of *L. hexandra* on 10 DAI. a. 0.5 cm b. 7 cm c. 9 cm d. 14 cm

CONCLUSION

Gamma-ray irradiation to the stolon has the potential to induce variations in *L. hexandra*. The sensitivity of plants to gamma rays is indicated by the value of LD50 at a dose of 68.85 Gy and LD20 at a dose of 29.36 Gy, with the linear equation y = 1.02-7.56x. The gamma irradiation dose of 25 Gy is the best dose to produce higher plants than the control (wild type).

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Study of Integrated Pest Management Strategy on The Population of Fruit Flies (*Bactrocera* spp.) in Red Chili Cultivation (*Capsicum Annuum*)

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Abstract

The fruit fly Bactrocera spp. is the main pest other than Thrips in red chilies, which can reduce plant productivity by 30-60%, so that a specific method of handling this pest is needed. This study examines the application of conventional and Integrated Pest Management (IPM) strategies to fruit fly populations in red chili cultivation (*Capsicum annuum*). Observation of fruit fly population used the comparative method with methyl eugenol traps and incubation of infected fruit. Determination of the research sample based on purposive sampling method and analyzed using descriptive analysis. The test parameters were the fruit fly population indicated by the host's density and hosts' availability in the applied IPM and conventional treatments. The results showed that the fruit fly species encountered were dominated by *B. dorsalis* with a percentage of 98.18% and *B. carambola* 1.82%. The fruit fly population's fluctuation in IPM and conventional treatments were significantly different, as evidenced by a one-way variance test at a significance level of 99%. The population of fruit flies in the IPM concept was 547 flies, while the conventional concept was 1546 flies. The percentage of fruit fly population in red chili plants with IPM treatment was 48% smaller than conventional treatments.

Keywords: *Bactrocera* spp., IPM, Population, Red chilies.

INTRODUCTION

The fruit fly *Bactrocera* spp. is the main pest after thrips on red chili plants, which reduced plant productivity by 30-60% [1]. In the initial attack, the larvae of *Bactrocera* spp. shows no symptoms, looks healthy and intact from the outside. After a few days, the fruit will change color to yellowish-red, and when viewed from the inside, there is a larvae of *Bactrocera* spp. Red chilies were attacked by *Bactrocera* spp. result in the fruit not being harvested because it will be fall out before it can be harvested. *Bactrocera* spp. was also included in the quarantine pest to watch out for and become one of the obstacles in chilies production [2]. The technique for controlling *Bactrocera* spp. has been done by control using synthetic pesticides. Insecticides with various frequencies do not affect the level of fruit fly pests [3]. The application of insecticides in controlling fruit fly pests is not effective because the larval phase is in the fruit tissue.

One approach in managing pests in cultivated crops is to implement Integrated Pest Management (IPM). The IPM can be interpreted as a pest management strategy oriented towards prevention and control by integrating all compatible techniques based on ecological principles [4]. Pest control techniques widely developed in IPM strategies are habitat manipulation by combining several companion plants and applying botanical pesticides.

Plantations managed with IPM and conventional strategies affect pest populations. It was reported that the intensity of Aphid attack on conventional treatments was higher than the IPM treatment on red chili plants [5]. Other studies have also shown that the thrips population is higher in conventional treatment than in IPM treatment [6]. However, the application of the IPM strategy on the population of fruit fly pests in red chili plants is not yet known. Therefore, it is necessary to research IPM strategies on fruit fly populations in red chili cultivation. This study aimed to determine the population of fruit flies in red chili cultivation managed by the IPM strategy.

MATERIAL AND METHOD

The materials used in this research are large red chilies of the Imola variety, organic fertilizers, chemical fertilizers, botanical pesticides, synthetic pesticides, alcohol, *Turnera subulata*, Methyl Eugenol, fruit fly traps, and incubation boxes. This research was conducted in the area of the red chili cultivation center in Andongsari Village, Ambulu District, Jember Regency. The research was carried out on red chili cropping areas with integrated and conventional pest management (IPM) treatments. In IPM land, the management techniques applied are refugia planting and application of botanical pesticides. In conventional land, the management technique applied is the application of synthetic pesticides. The following is a table of the components of the management (Table 1).

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Table 1. Management technique components

Treatment Components	IPM	Conventional
Companion planting	*Turnera subulata*	---
Pesticide	Botanical pesticide (soursop, papaya, and neem leaf)	Abamectin, Dimethoate, Imidacloprid, Cypermethrin
Time of pesticide application	Once a week (start 21-67 days after planting)	Once a week or anytime

Population of Fruit Flies

This activity consists of two movements, namely taking samples using attractant traps and taking samples by incubating the affected fruit (Fig. 1). The attractant trapping was carried out by sampling using the Methyl Eugenol attractant trap, each trap was given 3-4 drops of ME on cotton and 1% formalin [7]. The trapping is installed in the morning at 06.00 - 09.00 West Indonesian Time. This sampling was carried out once a week during the generative phase with one-week intervals. The catch was collected, and the trapped fruit fly population was counted every week.

Figure 1. Attractant traps and Fruits incubation box

Incubation of infected fruit was carried out by taking red chili fruit samples by purposive sampling with criteria of approximately 15-17 cm long with a diameter of 1.4-1.6 cm, reddish-green to red in color. This sampling was carried out once a week during the generative phase (10-19 WAP). As many as 20 pieces of fruit were taken each week on different plants, which were then incubated. The fruit flies and parasitoids that came out of the incubator were then collected, and the population counted.

Identification of Fruit flies

Identification was carried out at the Agrotechnology Laboratory, Faculty of Agriculture, University of Jember. Observations were made using the identification of fruit fly [8].

RESULT AND DISCUSSION

Population of *Bactrocera* spp.

Population of *Bactrocera* spp. obtained from traps and incubation of infected fruit every week of observation showed that the population of *Bactrocera* spp fluctuated and increased at each stage of plant age growth. The lowest population was found at 10 weeks after plantation (WAP) observations, while the highest population was found at 19 WAP observations. The chili plants aged 10 WAP entered the early harvest period of red chili plants so that the number of red chilies was still small and most of the fruit had not yet entered the physiological maturity phase, so the population of *Bactrocera* spp was still low. In the 19 weeks of observation, the availability of red chilies is in abundance. In addition, the data collection of infected fruit did not show any parasitoids that had appeared.

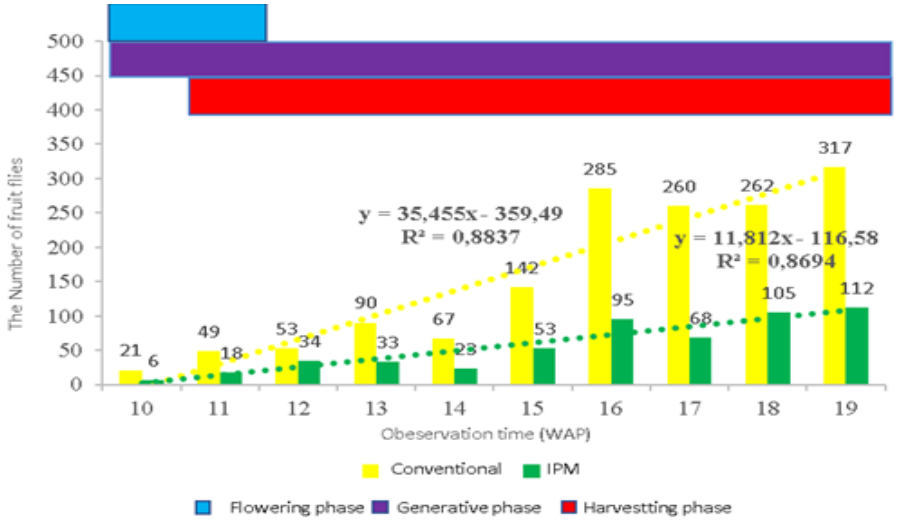

Figure. 2. Fluctuations of fruit flies' population in IPM and conventional treatments at each plant age development

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The results of observations of the fruit fly population at each plant phenology development showed that the high number of fruit fly populations in the field started from 10-19 WAP and continued to increase in each phase, especially in the 14th phase of the WAP, which entered the harvest phase. The population of *Bactrocera* spp. in these two treatments continued to increase with the plant's age (Fig. 2). The increase in plant age is related to the increase in the number of chilies. It is in line with the number of harvests per week, which continues to increase up to 20 WAP. The population of *C. capitata* is fundamentally influenced by the abundance and level of fruit maturity [9]. Besides, the presence of host plants and availability of hosts is one of the main factors for fruit fly populations [10].

Based on table 2, the total number of fruit flies in conventional treatment was higher and significantly different from the IPM treatment. One of the IPM strategy components is planting companion plants, namely *T. subulata* as a border. One form of the polyculture planting system is companion planting. Planting of *T. subulata* in oil palm plantations could reduce pest populations and attract natural enemies such as predators and parasitoids. *T. subulata* around oil palm plants can increase the number of parasitized pests so that the pest population density can be reduced [11,12].

Various research results indicate that the intercropping cropping pattern is effective in reducing pest attacks in the agroecosystem. Volatile compounds of cultivated and non-cultivated plants could inhibit pest behavior in finding host plants and reduce the rate of attack by these pests [13]. Companion plant planting can affect the pest population in an ecosystem. The intercropping of potatoes and celery could reduce Trips by 44% and *Myzus persicae* aphids by 55.6% on potato crops [14]. The factors that make cultivation vulnerable to pest attack include a decrease in landscape and plant diversity, pesticides, unbalanced fertilization, and climate change [15].

The population percentage of *Bactrocera* spp. in the red chili plantations that were treated with IPM is 26%, while in the conventional treatment, it was 74% (Fig. 3). Companion planting between the main crop and refugia can interfere with the discovery of host sites by pests, draw pests from protection targets, repel pests, cover the main crop, and camouflage the main crop or physically deter pests [13].

Table 2. The total population of fruit flies in each treatments

No	Treatments	number of fruit flies (head)
1	IPM	547 b
2	Conventional	1546 a
	P – value	0.009

Note: Numbers followed by different letters in the same column show a significant difference in the t-test = 1%

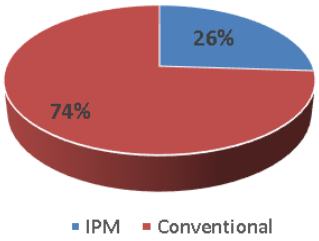

Figure 3. Percentage of total fruit flies' population in each IPM and conventional treatment

In addition to the influence of companion plants, the effect of botanical pesticide application is assumed to affect fruit fly populations on IPM treated land. It is because botanical pesticides can be used, among others, as a pest control agent, which kills pests quickly, acts as a substance that inhibits the development of insects or pests, and acts as an attractive agent, repellent substance, and food inhibitor. Botanical pesticides include plants matter (refined extraction), which can function as a killer, binding agent, and inhibitor of plant pests' growth [16]. Botanical ingredients were used soursop leaves and *gadung* tubers (*Dioscorea hispida*), which act as insecticides, larvacides, repellents, and antifeedants. Soursop leaves contain acetogenin compounds, including asimycin, roundacin, and squamosin, which function as pest repellents and anti-food items [17]. Soursop leaf extracts starting at a concentration of 2.5% have anti-eating activity and reduce the relative consumption rate and the relative growth rate of *S. litura* instar V [18].

Percentage of fruit fly species

The fruit fly species found in the red chili fields in Andongsari Village are *Bactrocera dorsalis* and *B. carambola*, 98.18% and 1.82%, respectively (Fig. 4). *B. dorsalis* dominated the dominant fruit flies in red chili cultivation in Bandung Regency at 93%. *B. dorsalis* is the main pest of red chilies and dominates other fruit fly species [19].

The dominant population of *B. dorsalis* is because this insect is invasive and competitive

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with other fruit flies and its host range is quite broad so that it becomes the dominant fruit fly species in cultivated crops, especially horticultural crops [20,21]. *B. dorsalis* has high reproductive power, wide distribution, high roaming ability, and polyphages [22]. In Indonesia, the fruit fly *B. dorsalis* (sin. *Bactrocera papayae*) is reported to attack chili plants, either *Capsicum annuum* or *Capsicum frutescens* [23,24].

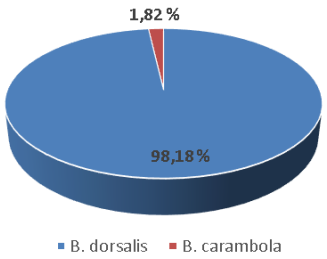

Figure 4. The percentage composition of the fruit fly species *B. dorsalis* and *B. Carambola*

CONCLUSION

The results showed that the fruit fly species encountered were dominated by *B. dorsalis* with a percentage of 98.18% and *B. carambola* 1.82%. Fruit fly populations in IPM and conventional treatments were significantly different as evidenced by a one-way variance test at a significance level of 99%. The population of fruit flies in the IPM concept was 547 flies, while the conventional concept was 1546 flies. The percentage of fruit fly population in red chili plants with IPM treatment was 48% smaller than conventional treatment.

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Minnow Trap Color Effectiveness Test Using Cat Food Bait as Aquatic Sampling Gear on Diurnal Fish in Gajah Mungkur Reservoir, Cental Java, Indonesia

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Abstract

The Minnow Trap is a simple sampling gear, efficient, easy to operate, affordable, easy to make, and has been used in research in the fisheries and aquatic ecology for more than 90 years. The brightness, color, and visual obstruction of fishing gear or fish traps affect the behavioral response of target fish, considering that each fish has a specific level of color sensitivity. The color effectiveness test of fishing gear is an important prerequisite for the use of sampling tools, and it needs to be evaluated to understand the gear performance and avoid potential sampling bias. The purpose of this study was to test the color effect of the minnow trap on diurnal fish in the Gajah Mungkur Reservoir waters. The research was carried out in Gajah Mungkur Reservoir, Wonogiri Regency, at two stations. The sampling conducted at 09.00 – 11.00 and 13.00 – 15.00 Indonesian West Time, where the initial survey is estimated to be the highest time for diurnal fish activity. The minnow trap used in this study is four colors vinyl-painted double funnel cylindrical minnow trap with a 5x5 millimeter mesh size and a conventional umbrella minnow trap used as control. The sampling results are then recorded on the datasheet and analyzed using Microsoft Excel. CPUE (Catch Per Effort Unit) of each unit is then calculated and statistically analyzed by Kruskal-Wallis test and Kolmogorov-Smirnov normality test through SPSS 25.0 software. The test result of five unit minnow traps from 40 installations and two hours of deployment time, 80 individuals were captured, consisted of seven species of freshwater fish. CPUE values ranking consecutively from the highest from silver units (1.375), black (1.25), green (1.125), red (0.9375), and control (0.3125). The Kruskal-Wallis analysis showed that all tested units do not have a significant difference.

Keywords: Color, catch per unit effort, fish, minnow trap.

INTRODUCTION

A minnow trap is a small fish trap with a funnel-shaped entrance on the side of a box or a cylinder-shaped net. The standard minnow trap design is often used for fish or crustacean sampling to collect aquatic ecological data. Minnow traps are classified as passive sampling devices because it rellies on fish to actively find and are interested in entering the trap [1]. The efficiency and selectivity of small fish traps are influenced by the probability that fish will meet, enter, and hold in the trap until they are taken [2]. The term *minnow* is used as a general term referring to small fish used as fishing bait. However, the true minnow taxonomically is given to freshwater fish that belong to the Cyprinidae family, which includes small species and juvenile schooling. The term minnow also applies to fish from the Poeciliidae family, such as Guppy, Molly, Platy, and Swordtail [3]. The size of fish caught in a minnow trap is limited by the size of the entrance, which is usually relatively small (20-30 mm). The Minnow traps can be effectively used to catch small freshwater fish samples in various wetland environments, including lakes, swamps, rivers, and ponds. The trapped fish samples are not damaged and can be released alive after being caught. Gill nets or angling methods usually cause mortality on fish, and predation also occurred in large-scale passive gear traps such as Fyke Net. Because of their small size, minnow traps can also be installed between complex habitats and small water pools or narrow waterways [4].

Minnow trap has been used as sampling gear in ichthyology and freshwater ecology studies for more than 90 years [5]. The design of the minnow trap is relatively simple, efficient, easy to operate, less expensive, and has been widely used for freshwater organisms sampling. Research and literature on the effectiveness of trap minnow traps have been studied in previous research regarding color effectiveness [6,7]. But there has never been researched on the use of minnow traps in tropical freshwater in Indonesia. Statistical test on the effectiveness and selectivity of fishing gear is essential to be measured for the use of a sampling tool when the data are intended for quantitative comparison [8]. Catch efficiency of various colors of fish traps needs to be evaluated to avoid potential sampling bias because the brightness

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and visual obstruction of the fishing gear affect the response behavior of the target fish [9].

Fishing gear visibility underwater depends on water turbidity and contrasts with the background of the habitat, which can be affected by seasonal changes in water turbidity color due to dissolved sediment or eutrophication. Andreev [10] recommends using darker nets or traps in clear water but using brighter colors in turbid water. Fish can distinguish colors, and different colors of fishing gear can determine the target. Fish retina generally has rod and cone cells. Fish vision is mediated by four visual pigments that absorb various wavelengths of light. Each pigment is constructed from chromophore and transmembrane proteins, known as opsins. Mutations in opsin have allowed for a wide range of variations in the absorption of wavelengths [11].

Some types of fish can see ultraviolet, and others are sensitive to polarized light. For example, Neumeyer [12] showed that minnows (*Phoxinus laevis*) were able to distinguish red, yellow, green, and blue colors from their irradiation levels. It shows that the minnows of the family Cyprinidae have excellent color vision on a par with mammals. Another study in 2017 also found the ability to see an even wider range of colors in the cichlid family [13]. These specifications lead to the idea that further research is necessary to determine the pattern of target sensitivity on trap color. This study is expected to be a reference in the standardization of fish sampling methods using minnow traps, especially in freshwater aquatic habitats in Indonesia.

MATERIALS AND METHODS

This study was conducted by experimenting with a sampling method using minnow traps by coloring treatment as an independent variable. The minnow trap that used in this study is a double funnel cylindrical minnow trap with 40 cm length, 30 cm funnel diameter, and 7 cm funnel entrance. The material of the trap is mesh wire coated with vinyl paint (red, silver, black and green) with a mesh size of 5x5 mm (Fig.1). The control of this experiment is foldable six funnels Umbrella Minnow Fish Trap with 100x100x40 cm and 2x2 mm mesh size. The purpose of using different shapes of minnow trap as control is to compare double funnel minnow trap with foldable umbrella minnow trap conventional sampling gear.

The research was conducted at Gajah Mungkur reservoir, Wonogiri district. The sampling process is conducted at two predetermined sampling stations:

- Sendang (-7.845784, 110.920640)
- Gumiwanglor (-7.876840, 110.894266)

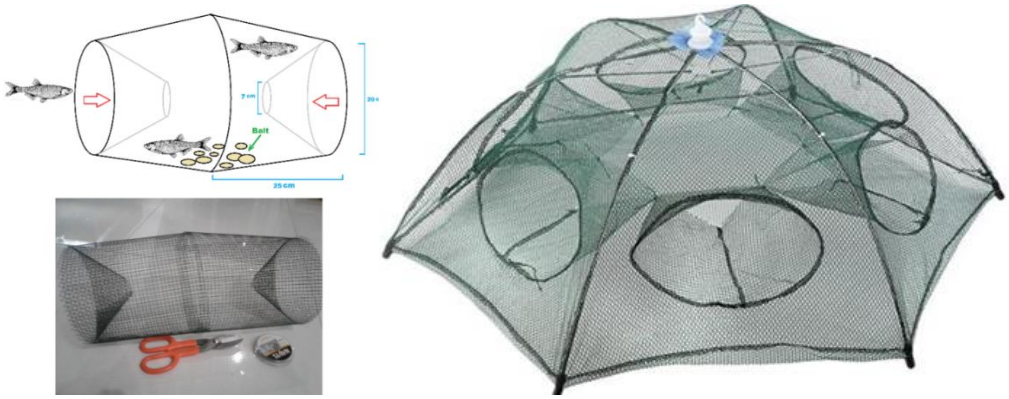

Figure 1. Double funnel cylindrical minnow trap and portable umbrella minnow trap

Site Description

This research was conducted in Gajah Mungkur Reservoir to test the effectiveness and selectivity of minnow traps. Gajah Mungkur Reservoir is an artificial lake or dam located at an altitude of 190 above sea level, an area of 8,800 ha (almost 90 km^2) with a maximum depth of 136 meters, which is located 7 km south of Wonogiri City just downstream of the Keduang River, Wonogiri Regency, Province Central Java. Gajah Mungkur is a multipurpose reservoir with the main function of irrigation, hydroelectric power, drinking water sources, tourism, fishing spot, and aquaculture. Gajah Mungkur Reservoir was built by damming the longest river in Java, the Bengawan Solo River. Gajah Mungkur Reservoir has six inlets (Keduang stream, Wiroko stream, Wuryantoro stream, Temon stream, Alang - Solo Hulu stream, and Uplahan stream) and one outlet (Fig. 2).

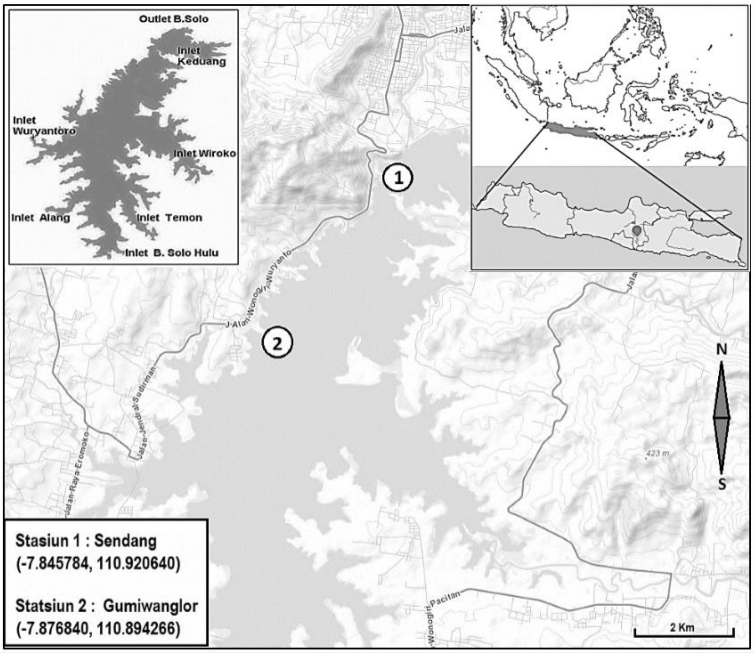

Figure 2. Study site in Gajah Mungkur Reservoir, Central Java

Data Collection

Survey and pre-sampling are necessary to decide the sampling station and estimate effective trap placement duration and escaping rate. Pre-sampling conducted by trapping at several durations (2 hours, 4 hours, and 6 hours) using the same unit. Habitat characteristics observation is carried out as consideration for selecting stations, including slope level, the dominant substrate (boulder, cobble, gravel, sand, clay, or mud), waters color (clear, cloudy, eutrophic), and aquatic vegetation presence.

Table 1. The composition and nutritional value of the used bait.

Composition	Nutrition Information
Tuna extract	Protein (min) = 28%
Fish meat	Fat (min) = 8%
Chicken meat	Rough fiber (max) = 3%
Wheat	Water concentration (max) = 10%
Corn	
Flour	
Whole soybean	
Taurine	
Omega oil	
Choline chloride	
Niacin	
Inositol	
Multivitamin (AD3, E, C, B1, B2, B6, B12, K3)	
Biotin	
Folic acid	
Dissolved minerals	
Yucca schidegra extract	

Source: PT. Matahari Sakti

Sampling was conducted by placing all trap units by four repetitions in each station. The traps were placed in the *photic* zone, approximately 0.5 – 1 meter (according to the bank slope level). The trapping duration of each placement is two hours at 09.00 – 11.00 and 13.00 – 15.00 West Indonesian Time. The bait that used in this study is dry cat food with the consideration that it contains the main nutrients (protein, fat, and carbohydrates) as well as vitamins and minerals (Table 1). Relatively larger granules also make dry cat food easier to apply to the size of the Minnow Trap mesh size. The captured specimens were then contained in the icebox and identified in the laboratory.

Data Analysis

Analysis using Catch per Unit Effort (CPUE) in this study is used to measure the effectiveness of each minnow trap color (silver, green, black, and red). The efficiency of the fishing gear for the sampling method in the study of water science and ecology can be represented by the ability of the sampling gear to catch fish and other aquatic biotas in a unit of time and repetition called CPUE. The total catch of each fishing gear unit that is tested will be divided by the number of repetitions and time of placement. Furthermore, for being able to measure the efficiency of the sampling method, the CPUE measurement of fishing gear can also be carried out to monitor and estimate the population of an aquatic species when combined with the recapture

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method [14]. The CPUE scale that is used in this study is individuals/units/hour.

$$\boldsymbol{CPUE\ i} = \frac{Total\ Catch}{Effort}$$

Description:
CPUE i = *Catch Per Unit Effort* unit *i*
Total Catch = Total traped organism by unit *i* (by an individual)
Effort = Total number unit trap *i x* repetition *x* replacement periods (hour)

The Kruskal-Wallis statistical tests and Kolmogorov-Smirnov normality tests used in this study followed with Mann-Whitney post hoc test with hypotheses:

- **H_0**: There is no significant difference between the average catch of minnow trap red, silver, blue, and green minnow traps
- **H_1**: There is a significant difference between the average catch of the red, silver, blue, and green minnow traps

RESULT AND DISCUSSION

Catch Per Unit Effort (CPUE)

The total catch was from 40 sampling processes of 5 unit minnow traps from 40 installations and two hours of deployment time of four colors cylindrical minnow trap units and one portable umbrella minnow trap as controls. We obtained 80 specimens from four families that included six fish species and one freshwater shrimp species. The results of research sampling show that the highest CPUE was possessed silver units (1.37 individuals/hour), followed by black units (1.25 individuals/hour), green (1.12 individuals/hour), and red (0.93 individual/hour). The smallest CPUE is possessed by an umbrella trap as control (0.31 individual/hour). The umbrella minnow trap as a control unit has the lowest CPUE. It was estimated because of a larger funnel hole that increases the escaping rate of the captured specimen. Umbrella minnow trap also more effective with longer placement duration according to the previous study with 4-6 hours duration [15], compared with this study which only 2 hours duration for each placement.

Invasive Nile Tilapia (*O. niloticus*) is the highest captured species by minnow traps Total catch was dominated by Cyprinid fish but also consisted of freshwater shrimp and one Goby species. The minnow traps are very effective in habitats that dominated by small Cyprinidae families. An experimental study conducted by Paradis and Dupuch [6] recommended using silver minnow traps because they show high capture ability due to the effects of transparency, and fish tend to enter black-colored minnow traps as a shelter in response to avoid predators. The maximum catch record is possessed by the red unit (8 individuals) followed by green (7 individuals), silver (6 individuals), black (5 individuals), and the smallest by umbrella minnow trap (4 individuals). The red unit in this study has the highest total catch. It assumes that fish tend to be less sensitive with long-wavelength light colors and less aware of trap visibility underwater [13]. The green unit has the second-highest total catch. It assumed that green color would give the trap camouflage on watercolor due to algal eutrophication or mimicking vegetation structure. Relatively lower CPUE of the red and green unit, even with the highest maximum trap, shows that fish tend to easier to enter the red and green trap and also easier to escape and caused unstable catch frequency.

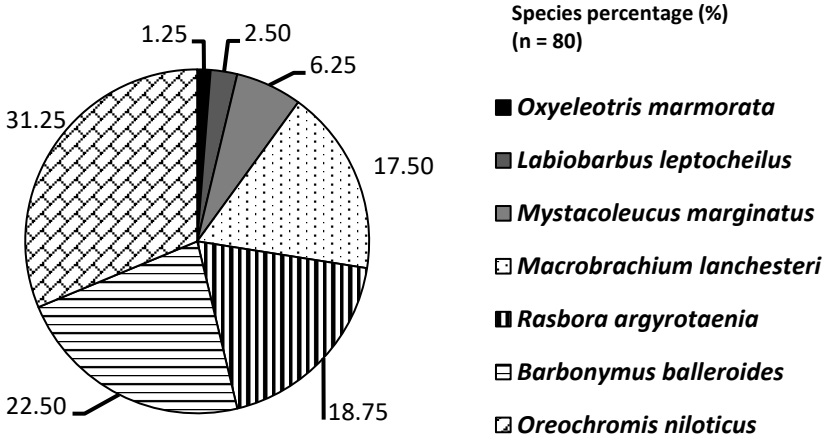

Figure 3. Total catch composition of all minnow traps.

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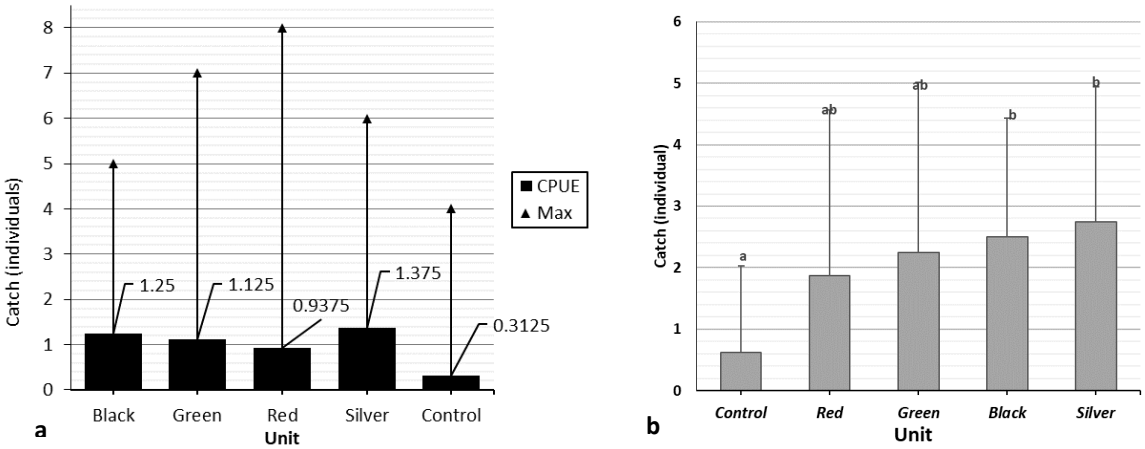

Figure 4. Comparison of a) CPUE value and maximum catch (2 hours placement) for each minnow trap unit b)The results of the post-hoc Mann-Whitney analysis of the minnow traps catch

The highest CPUE value in this study was relatively lower compared to the similar experiment by MacRae and Jackson [5] using the silver-colored minnow trap in a community of fish with moderate temperate lakes in the northern hemisphere (mean CPUE = 1.61). The effectiveness of the minnow trap conducted in the brackish waters of the Baltic Sea shows that the silver-colored minnow trap also has the highest CPUE value (2.8 individuals/unit/hour) compared to black and red [7]. Merilä [16] used minnow traps in Rytilampi lake Finland with the result of 1.31 individuals/units/hour for silver minnow traps and 0.20 individual/units/hour for black minnow traps. The CPUE values in this study have a wide range compared to the highest capture value (maximum capture) of each unit (Fig. 4a). The data shows the instability of the catch frequency at each placement.

Statistical Analysis

Statistical analysis on total catch shows that the data are not homogenous and don't have a normal distribution. Kruskal–Wallis analysis shows the insignificant difference (df=4, P>0.05) from 8 replications of 5 tested units unit minnow traps. Mann-Whitney Post Hoc test only shows the difference between unit control with black and silver rather than red and green units (Fig. 4b). The post Hoc test also shows that four units excluded from control don't have a significant difference since the umbrella minnow trap has the largest gap of total catch number with four double funnel minnow trap units. It gives double funnel minnow trap advantages that superior at shorter placement duration [7]. The performance of using minnow traps is not only influenced by the ability of fishing gear to attract fish to enter but also the ability to prevent fish from escaping the fishing gear. It is explained by a larger unit silver CPUE than the darker colored units, which have a bigger escaping rate via funnel holes visibility. The factors of the presence and density of fish communities also have a big influence on the effectiveness of fishing gear. An experiment conducted by Layman and Smith [4] showed that the size of freshwater habitats or water bodies directly increases the bias of the sampling method. Captured number fluctuation is also affected by target movement in large habitat and gives a disadvantage to minnow trap as passive sampling gear.

CONCLUSION

Five units of tested minnow trap in Gajah Mungkur Reservoir showed that the silver-colored minnow trap unit has the highest CPUE (1.375), followed consecutively by unit black (1.25), green (1.125), red (0.9375), and umbrella minnow trap as control (0.3125). The Kruskal-Wallis analysis showed that all tested units do not have a significant difference. The large margin of data deviations indicates a lack of sampling repetition, data fluctuation is also affected by habitat size. Future research using minnow trap recommended better using a larger number of trap units and avoiding the application of minnow trap on large water bodies habitats.

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Risk Prevention of Ventilator-Associated Pneumonia Thorough Oral Hygiene: *A Literature Review*

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Abstract

Ventilator-Associated Pneumonia (VAP) is a type of nosocomial infection that mostly occurs in intensive care units, generally occurring 48 hours after intubation. Endotracheal intubation and the use of a mechanical ventilator are invasive measures by patients, having a therapeutic effect. This paper aimed to identify the use of oral hygiene materials for the incidence of pneumonia related to the use of a ventilator. The research design was by searching literature in five databases, namely PubMed, Proquest, Science Direct, Wiley Online Library, and Google Scholar, by taking all databases in English and Indonesian, published in the last ten years (2010-2020). There were six articles about the effectiveness of using oral hygiene as risk prevention for Ventilator-Associated Pneumonia (VAP) with various concentrations and volumes. Several oral health assessment tools that can be used are Oral Assessment Guide, Beck Oral Assessment Scale, or Mucosal-Plaque Score. Comprehensive implementation of oral hygiene can improve the oral health of patients with mechanical ventilation, so bacterial colonization that causes VAP can be prevented. Therefore, nurses need to understand and apply oral assessment instruments as a basis for giving intervention.

Keywords: Intensive care unit, Oral hygiene, Ventilator-Associated Pneumonia

INTRODUCTION

The mortality and disability rates in critically ill patients who are admitted to the ICU were increasing from year to year. It is due to disease conditions, care, and nosocomial infections in the hospital. One of the most common nosocomial infections in the ICU is a complication of using a ventilator was Ventilator-Associated Pneumonia (VAP).

A ventilator is a breathing device to maintain optimal ventilation and maximize oxygen transport [1]. There have been many strategies that have been implemented to prevent the incidence of VAP. One of which is oral hygiene, the most important part of nursing interventions in intensive care. However, it is necessary to consider the negative effects that will be experienced by patients. Antibiotic resistance and risk of aspiration are some of the effects that can be fatal in patients [2], so it is necessary to find alternatives in oral care regimens.

Endotracheal intubation and the use of mechanical ventilators are invasive measures by patients who are treated in intensive care, but apart from having therapeutic effects, they can also cause side effects. It was Ventilator-Associated Pneumonia, increased morbidity rates around 24-70 %, and in the length of stay 9.6 days, thus significantly increasing the cost of care and treatment at the hospital [3].

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The patient condition in the care unit received several invasive measures, and the treatment caused an immunosuppressive and was prone to antibiotic resistance. The installation of an endotracheal tube causing the oral condition to become worse. The continuous open oral causes the mucosa to become dry, saliva production decreases because the mechanical function of the mouth does not play a role after all food is inserted through a nasogastric tube.

Nurses play a crucial role in performing oral hygiene interventions as measure prevention of the risk of VAP. Previous studies have widely explained that oral health is closely related to the incidence of VAP, with an incidence of up to 117 per 1000 ventilator days with 69 patients (42.6%) developing early-onset VAP at 0-48 hours of Endotracheal Tube (ETT) use [4].

The risk of VAP incidence can be prevented through maximum oral care by reducing bacterial and fungal overgrowth [5]. Good oral care will also significantly reduce plaque, salivary bacteria, and potential respiratory pathogenic cells [6], but the standards for oral hygiene in each hospital are different [7]. Interventions can be different according to the standard operating procedures of each hospital. Research revealed that chlorhexidine 0.12% is the golden standard of oral care because it has antibacterial and antiplaque effects [8].

This paper aimed to identify the effects of using oral hygiene materials on the incidence of pneumonia-related use of a ventilator as the primary outcome. The secondary outcome/point

of review including patient characteristics, instruments used, duration of time, method, frequency, and types of bacteria isolated, so that we can recommend alternatives for nurses in a clinical service.

MATERIAL AND METHOD

This study is a literature review by taking articles from all countries in English in the last ten years (2010-2020). In searching for articles, several databases are used, including Pubmed, Proquest, Science Direct, Wiley, and Google scholar. A list of references taken manually in the last ten years (2010-2020).

Searching articles using the keywords "oral care", "oral hygiene", "oral health care", "critical patient", "intensive care unit", "ventilator-associated pneumonia", and "VAP". If there is an inaccessible article, the author does not make any search efforts. Content analysis was not carried out if the samples were varied, the results and methods were different. For this literature review, the authors analyzed the data based on the similarity of oral hygiene interventions and their effects on oral health and colonization of types of oral bacteria.

RESULT AND DISCUSSION

The PubMed search results totaled 13 articles, ProQuest 14 articles, Science Direct 11 articles, Wiley 11 articles, Gray literature 15 articles, thus brought a total of 64 articles. The results were specified for publications in the last ten years (2010-2020) in English. Then the exclusion results, which is not a full text, non-experimental, and multiple articles were 30 articles. There 28 articles that were not accordance to the resulting study, so that only six articles were included for the final review.

Table 1. Description of Article Review

Objective	Respondents	Intervention	Results
Determine the effect of chlorhexidine plus toothbrushing gluconate oral rinse for preventing ventilator-associated pneumonia [9].	150	A dentist provided instruction and supervised oral hygiene to patients who underwent cardiac surgery and who received regular oral hygiene care.	Observed in group 1, there were no significant differences in all-cause in-hospital death between groups. A lower incidence of ventilator-associated and a shorter hospital stay. The risk of developing pneumonia after surgery was three times higher in Group 2.
Evaluate the efficacy and safety of oral care with povidone-iodine on the occurrence of ventilator-associated pneumonia in the high-risk population [10].	119	Participants were randomly assigned to received oropharyngeal care with povidone-iodine six times daily until mechanical ventilation withdrawal.	There was a significant difference between the groups for ventilator-associated tracheobronchitis: the povidone-iodine group and the placebo group.
Relationship between chlorhexidine 0.2% and povidone-iodine 1% on the incidence of VAP [11].	32	Subjects who met the inclusion criteria were divided into two groups, those who received chlorhexidine and povidone-iodine.	There was a relationship between povidone-iodine and chlorhexidine against the occurrence of ventilator-associated, but the relationship was not significant.
Comparing the effectiveness of oral hygiene using lactoperoxidase enzymes with chlorhexidine in VAP prevention [12].	127	Determined the group that to be used as an experiment, which using chlorhexidine and Oral Hygiene.	There was no significant difference in oral hygiene using Lactoperoxide and Chlorhexidine in the prevention of ventilator-associated pneumonia.
Effect of the implementation of oral hygiene using antiseptic hexanol gargle in minimizing the incidence of Ventilator-Associated Pneumonia (VAP) in R. ICU, Tugurejo Hospital [13].	15	The pre-test and the post-test data were taken on the first day and fifth day when the ventilator was installed. They used the same antibiotic and used SOP of oral hygiene and clinical pulmonary infection score (CPIS) observation sheet.	There was a significant difference before and after oral hygiene using hexanol gargle the incidence of ventilator-associated pneumonia (VAP).
Evaluate the effects of oral hygiene with hexanol and chlorhexidine in patients with mechanical ventilators in ICU [1].	30	The subjects were divided randomly into two groups: subjects given by hexanol 0.1% and given by chlorhexidine 0.2% twice a day.	There was no significant difference in oral hygiene with the use of hexanol and chlorhexidine.

Ventilator-Associated Penumonia (VAP)

Ventilator-Associated Pneumonia (VAP) is a type of nosocomial infection that mostly occurs in intensive care units, usually occurring 48 hours after intubation. VAP defines as a condition of the presence of a new infiltrate and permanent on the chest X-ray accompanied by one of the signs in the form of a blood or pleural culture similar to microorganisms found in sputum or tracheal aspiration, cavitation on chest radiographs according to the American College of Chest Physician. There are three symptoms, i.e. high fever, leukocytosis, and a type of nosocomial infection that most often occurs in intensive care [14]. It generally occurs after 48-72 hours after the endotracheal tube (ETT) insertion to patients who use mechanical ventilator support. If this occurs after the first four days of mechanical ventilation, then this VAP is considered early-onset, whereas late-onset occurs after the 5th day of mechanical ventilation.

Risk Factors

Risk factors incidence of VAP in the ICU, including the history of disease/comorbidity patient, such as the history of lung disease, smoking, the history of diabetes mellitus, and the suction method [5]. Besides age, gender, trauma, and the influence of antibiotic use, VAP is also closely related to patient oral hygiene [15]. Although a diagnosis of VAP is difficult to be established, its incidence increased inpatient length of stay up to 9.6 days. The increase in care and treatment costs for each patient is US$ 40,000, which implies a high mortality rate of 24-70% of patients in ICU [16].

Pathogenesis of VAP

Ventilator-Associated Pneumonia (VAP) occurs due to disruption of the body's defense system, especially in patients with decreased consciousness. The patient loses the ability to perform care and maintain oral hygiene. It causes mechanical functions of the mouth such as chewing, biting food, and swallowing have decreased. The natural defense system cannot function properly, causing the accumulation of bacteria in the oropharynx, bronchi, and trachea [17]. Saliva production has also decreased [18]. There is a buildup of plaque on teeth and biofilms on the tooth surface, which is beneficial for bacterial growth and colonization.

According to the Clinical Practice Guideline for Hospital-Acquired Pneumonia (HAP) and VAP in adults [19], this infection occurs when the patient is admitted to the ICU and uses mechanical ventilation for more than 48 hours, with an increased risk of incidence 3-10 times with a fairly high mortality rate between 24-50%. It can reach 76% under certain conditions when compared to patients who are not on a ventilator.

Caused of VAP

Some bacteria as the cause of VAP are generally gram-negative bacteria, including; *Pseudomonas, Klebsiella pneumonia, Enterobacter, Serratia* spp, and *Acinetobacter* spp. The diagnosis of VAP is established using the CPIS assessment instrument, includes purulent bronchial secretions, leucopenia < 1000.mm^{-3} or leucocytosis > 12,000.mm^{-3}, increased body temperature > 38° C or < 36° C without cause, positive blood culture, and visible infiltrates on chest x-ray. If three of these symptoms occurred in an intubated patient, a VAP can be diagnosed [14].

Prevention of VAP

Among the VAP prevention strategies, oral care is the most crucial part for nurses in the ICU to maintain oral health and prevent the colonization of pathogenic bacteria that cause pneumonia. It stated in a study that there is a strong correlation between unhealthy oral conditions and an increased incidence of VAP [20].

Oral Hygiene

Oral hygiene is the act of cleaning the oral cavity, teeth, and tongue. This action is one of the essential interventions in intensive care, which affects patient care and recovery [20]. Prioritizing oral hygiene for patients in critical care can prevent complications that will aggravate the patient's condition. It is very important to maintain the integrity of the lips, tongue, and oral mucosa. Thus, routine oral hygiene can prevent infection of the oral cavity and moisturize mucous membranes of the mouth and lips.

Impact of Oral Hygiene

Some of the oral hygiene that is not optimal causes periodontal infections to occur, trauma to the gums, moldy/white tongue, stomatitis, gingivitis, discoloration of teeth, and caries. In critical patients, the natural defense function is impaired, making it easier for infection to occur in the respiratory tract. If no action is taken in prevention, it causes increased bacterial colonization, and bacteria can translocate to the

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lower respiratory tract, which triggers pneumonia (ventilator-associated pneumonia).

Several ingredients are often used for oral hygiene, including normal saline, chlorhexidine, povidone-iodine, toothbrush and toothpaste, as well as honey. Some studies explained that added chlorhexidine to oral care will reduce infection-causing bacteria, as well as a broad-spectrum antibacterial, which will reduce the incidence of ventilator-associated pneumonia [21]. Some side effects of chlorhexidine have been reported, like dry oral mucosa, tooth staining, taste changes, and the long-term effect is resistance to certain bacteria [22].

CONCLUSION

The main intervention for the prevention of oral infections in patients on mechanical ventilators is oral hygiene. The mouth is the main gateway for bacteria that cause infection. Therefore, oral hygiene needs to be considered comprehensively by nurses who are in charge of intensive care. The frequency of implementation of oral hygiene is adjusted to the results of the oral health assessment of each patient. All of these components are intended to prevent the colonization of microorganisms, so the incidence of VAP in patients in intensive care rooms with mechanical ventilation can decrease.

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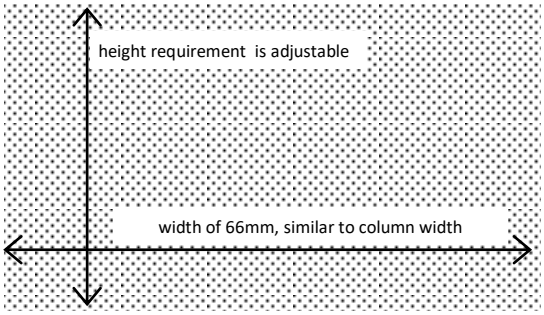

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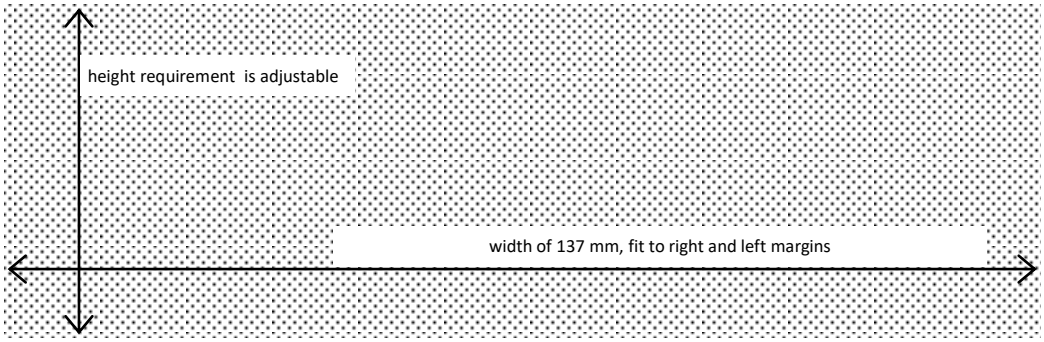

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