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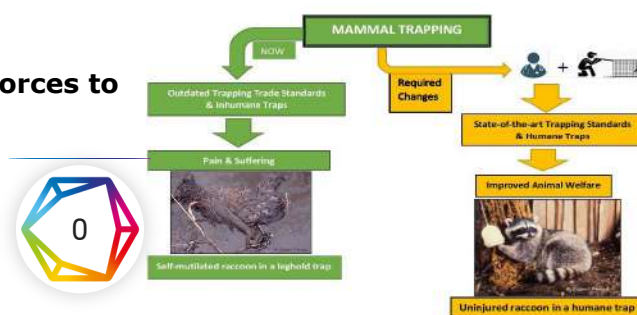
Short Communication

Veterinarians and Wildlife Biologists Should Join Forces to End Inhumane Mammal Trapping Technology.

Proulx G.

World Vet. J. 11(3): 317-318, 2021; pii:S232245682100043-11

DOI: <https://dx.doi.org/10.54203/scil.2021.wvj43>



ABSTRACT: Current mammal trapping standards uphold the use of inhumane trapping technology. For example, killing neck snares for the capture of canids, and rotating-jaw traps, and steel-jawed leghold traps for procyonids and mustelids, are being used by trappers despite decades of research showing that they are inhumane, and cause serious injuries and distress in captured animals. Many wildlife biologists unsuccessfully raised concerns about inhumane mammal trappings. This short communication stresses the need for veterinarians and wildlife biologists to work together to improve the fate of mammals captured in killing or restraining traps, and modify mammal trapping standards on the basis of animal welfare science.

Keywords: Humaneness, Mammal trapping, Traps, Trapping standards

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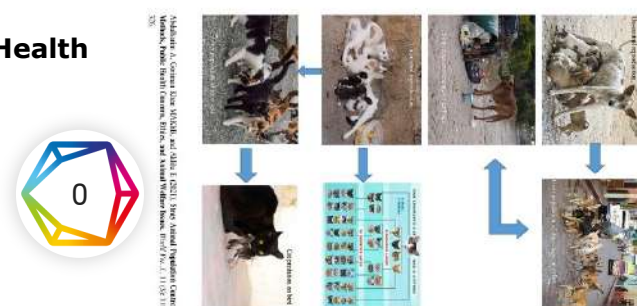
Review

Stray Animal Population Control: Methods, Public Health Concern, Ethics, and Animal Welfare Issues.

Abdulkarim A, Goriman Khan MAKhB, and Akililu E.

World Vet. J. 11(3): 319-326, 2021; pii:S232245682100044-11

DOI: <https://dx.doi.org/10.54203/scil.2021.wvj44>



ABSTRACT: Stray animal overpopulation has become one of the most serious global problems with many negative impacts on the community, environment, and public health. Most of the stray animals do not depend on humans for food and shelter, and therefore, can reproduce uncontrollably. The uncontrolled reproduction of stray animals increases their population that leads to a higher chance of predation, road traffic accidents, transmission of zoonotic diseases, and therefore, becoming vectors for some diseases. There are several methods for stray animal population control depending on the situation and the nature of the stray animals. These methods include contraceptives, trap-neuter-return, poisoning, euthanasia, and gun shooting among others. Each of the outlined methods has its advantages and disadvantages as to their practicality, ease of conducting, cost, effectiveness, ethics, and animal

welfare issues. In conclusion, to achieve successful control measures of the stray animal population and the problem they create, the concerned authorities need to design and enact animal rights laws, provide medical care (treatment and vaccination), feeding, shelter for the animals, and control their reproduction. Public health and environmental agencies may improve the services by regulating personal and environmental hygiene, prevention, and control of zoonotic and transmissible diseases that can be transmitted from stray animals to the public and other livestock respectively.

Keywords: Animal welfare, Ethics, Population control methods, Public health, Stray animals

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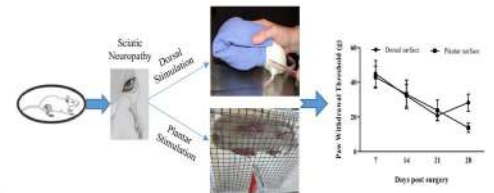
Research Paper

Comparison of Painful Response to Mechanical Stimulation of the Plantar and Dorsal Surface of Paw Following Chronic Constriction Injury-induced Neuropathic Pain.

Ghanbari A and Mohammadi M.

World Vet. J. 11(3): 327-332, 2021; pii:S232245682100045-11

DOI: <https://dx.doi.org/10.54203/scil.2021.wvj45>



Ghanbari A and Mohammadi M (2021). Comparison of Painful Response to Mechanical Stimulation of the Plantar and Dorsal Surface of Paw Following Chronic Constriction Injury-induced Neuropathic Pain. *World Vet. J.*, 11 (3): 327-332.

ABSTRACT: Mechanical and thermal stimuli were used to evaluate neuropathic pain-like behavior in animal models usually. Mechanical stimulation of paw plantar surface is commonly used to determine mechanical allodynia. In the present study, paw withdrawal response to plantar surface stimulation was compared with paw withdrawal response to dorsal surface stimulation. To this end, a total of 30 female Wistar rats (180-220 g), were assigned randomly to three groups as intact (without any manipulation), sham (incision of skin and muscles without nerve injury), and neuropathy (sciatic nerve lesion) with 10 in each group. To induction of neuropathy (chronic constriction injury), four movable ligations were established around the sciatic nerve using catgut chromic suture with a distance of one millimeter apart and then wound incision was closed. In the sham group, the incision site was closed without nerve ligation. Mechanical allodynia was examined by Von Frey filaments for four weeks. The findings indicated that the paw withdrawal threshold following dorsal surface stimulation was significantly reduced compared to the sham group at day 21 post-surgery. Moreover, paw withdrawal threshold following plantar surface stimulation significantly decreased compared to the sham group at day 21 post-surgery. The present results regarding the sham group showed that the paw withdrawal threshold after mechanical stimulation of the plantar surface was not significantly different from that of the dorsal surface paw. In addition, and there was no significant difference between the paw withdrawal response to plantar surface and dorsal one. In conclusion, paw withdrawal threshold to plantar surface mechanical stimulation was not significantly different from one in dorsal surface following neuropathic pain induced by chronic constriction injury.

Keywords: Mechanical allodynia, Neuropathic pain, Paw dorsal surface, Paw plantar surface, Rat

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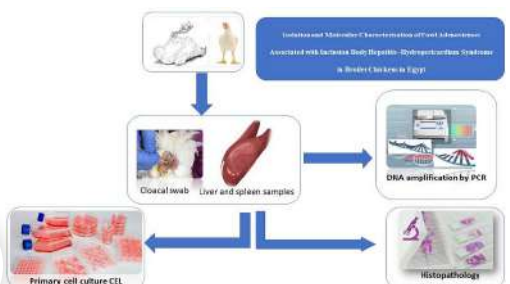
Research Paper

Isolation and Molecular Characterization of Fowl Adenoviruses Associated with Inclusion Body Hepatitis-hydropericardium Syndrome in Broiler Chickens in Egypt.

Al Naguib MG, Morsy EA, Samir M, Khattab MS, Khelfa DG, and Abdel-Alim GA.

World Vet. J. 11(3): 333-342, 2021; pii:S232245682100046-11

DOI: <https://dx.doi.org/10.54203/scil.2021.wvj46>



Al Naguib MG, Morsy EA, Samir M, Khattab MS, Khelfa DG, and Abdel-Alim GA (2021). Isolation and Molecular Characterization of Fowl Adenoviruses Associated with Inclusion Body Hepatitis-hydropericardium Syndrome in Broiler Chickens in Egypt. *World Vet. J.*, 11 (3): 333-342.

ABSTRACT: Avian adenoviruses are an extremely diversified group of pathogens that recently triggering a variety of problems for poultry production. In particular, Inclusion Body Hepatitis-Hydropericardium Syndrome (IBH-HPS), which has been observed in broiler birds from 3 to 6 weeks of age and is associated with anemia, hemorrhagic disorders, hydropericardium, and high mortality. The disease has been reported worldwide, and recently it was reported in many Egyptian farms, causing severe economic losses. Therefore, the current study aimed to isolate, and genetically type the most common Adenovirus serotypes associated with this syndrome in Egyptian farms. A total of 50 broiler chicken farms (3-6 weeks old) located in different Egyptian governorates were examined. Macroscopically, the diseased flock revealed hydropericardium, enlarged friable livers with ecchymotic hemorrhages, and varying mortality rates (1 to 7.5%). Histopathologically, severe diffuse necrotizing enteritis, hepatitis, pericarditis, and diffuse lymphoid depletion of the spleen were the most prominent lesions. Liver tissues and cloacal swabs were collected from all examined flocks for FAdVs detection by conventional polymerase chain reaction (PCR) targeting the L1 loop in the hexon gene. The PCR products were sequenced for typing of the detected viruses. It was found that 10 out of 50 flocks examined were PCR positive for FAdVs (20%). Phylogenetic analysis of the sequenced genes revealed that the obtained viruses clustered with reference strains belonging to FAdV type D and E serotype 2, 11, and 8a respectively. The isolation of both FAdV type D and FAdV type E were carried out on a primary cell culture chicken embryo liver cell (CEL) and the presence of these viruses was confirmed by PCR after the appearance of cytopathic effect (CPE). From this study, it could be concluded that both FAdVs types D and E are the most common adenoviruses circulating in poultry farms suffering from hydropericardium and inclusion body hepatitis.

Keywords: Broiler chicken, Chicken embryo liver cell, Fowl adenovirus, Hexon gene, Histopathology, Inclusion body hepatitis-hydropericardium syndrome, PCR

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Research Paper

Molecular Identification of *Trypanosoma theileri* and Biology of Trypanosomes.

Fentahun T and Paeshuyse J.

World Vet. J. 11(3): 343-367, 2021; pii:S232245682100047-11DOI: <https://dx.doi.org/10.54203/scil.2021.wvj47>Fentahun T and Paeshuyse J (2021). Molecular Identification of *Trypanosoma theileri* and Biology of Trypanosomes. *World Vet. J.* 11 (3): 343-367.

ABSTRACT: *Trypanosoma theileri* (*T. theileri*) is a non-pathogenic, cosmopolitan, and commensal protozoa of cattle. The main objective of the current study was to investigate the biology and feasibility of *T. theileri* as a model candidate for the discovery of a novel drug. In the present study, the isolates of *T. theileri* obtained from the Institute of Tropical Medicine (ITM) in SDM 79 were cultivated at 26°C. Eight experiments with different inoculum and different times were grown. The growth curve was plotted to check the growth trends. The doubling time in the logarithmic phase was determined to be 17.43 hours. In addition, an experimental infection was done on a 3-month-old Holstein Friesian calf to isolate the blood-streaming shape; however, it was not successful after the blood buffy coat smear and PBMC culture in RPMI 1640 and HMI 9. Furthermore, the viability was determined by quantitative colorimetric Resazurin assay in 96-well fluorescence Microplates containing 0.4 to 2.4 mM of Resazurin. On the other hand, the response to Pentamidine (1-100 ng/mL) showed a strong negative correlation between the fluorescence signal and the highest Pentamidine concentration. IC₅₀ was 9.25 ng/mL. Genomic DNA was extracted using the phenol-chloroform method. The gradient PCR amplification using *T. theileri* specific PCR (Tth625-PCR) primers was detected at 465 base pair (bp). In addition, the full-length 18S rDNA sequence was detected at 730 bp. In the silico analysis using common anti-trypanosome drug targets, no significant similarity could be found on either the DNA or the protein level. Nevertheless, homologous sequences have been identified among the drug targets for Ornithine decarboxylase. Therefore, the analysis might show the possibility of using *T. theileri* as a model for the search of new drugs once they have entire genome sequences. Analysis of the whole genome and transcriptome indicated a phylogenetic relationship between *T. theileri* and other pathogenic trypanosomes which can be the basis for novel drug development.

Keywords: Drug model, Novel drug, PCR, Resazurin, SDM 79, *Trypanosoma theileri*

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Research Paper

Immunolocalization of Steroidogenic Enzymes (3 β -hydroxysteroid dehydrogenase, 17 β -hydroxysteroid dehydrogenase, and P450scc) in Rats with Testicular Dysfunction Treated with Mesenchymal Stem Cells-conditioned Medium.

Khasanah LM, Budipitojo T, and Fibrianto YH.

World Vet. J. 11(3): 368-376, 2021; pii:S232245682100048-11DOI: <https://dx.doi.org/10.54203/scil.2021.wvj48>Khasanah LM, Budipitojo T, and Fibrianto YH (2021). Immunolocalization of Steroidogenic Enzymes (3 β -hydroxysteroid dehydrogenase, 17 β -hydroxysteroid dehydrogenase, and P450scc) in Rats with Testicular Dysfunction Treated with Mesenchymal Stem Cells-conditioned Medium. *World Vet. J.* 11 (3): 368-376.

ABSTRACT: About 60-80 million couples in the world are suffering from infertility disease. Infertility is a major problem in patients coping with chemotherapy. The chemotherapy process can degenerate non-target organs, especially in testes. Infertility in male or testicular dysfunction is caused by the failure of proliferation and differentiation of the spermatogenic cells. Many studies reported that mesenchymal stem cells-conditioned medium promoted regenerative processes. The present study aimed to investigate the effect of mesenchymal stem cells-conditioned medium on the cisplatin-induced testicular dysfunction by examining the immunolocalization of steroidogenic enzymes, such as 3 β -hydroxysteroid dehydrogenase, 17 β -hydroxysteroid dehydrogenase, and P450scc which are considered as markers of steroid production. All experimental animals were divided into three groups, namely the control group, mesenchymal stem cells-conditioned medium treated group with an injection dose of 0.2 ml/kg body weight (BW, P1), and mesenchymal stem cells-conditioned medium treated group with an injection dose of 0.5 ml/kg BW (P2). Cisplatin was injected into both treated groups to induce testicular dysfunction. The testicular tissues were processed by the paraffin method, then cut to a thickness of 5 μ m, followed by immunohistochemical staining. The HSD3B1 immunoreactivities were found only in Leydig cells, and the intensity increased every week after the injection of mesenchymal stem cells-conditioned medium. The variety of weeks and groups was significantly different in the number of immunoreactive cells of HSD3B1. The results indicated a significant difference between one week after the first injection and the one week after the third and fourth injection. The findings showed a significant difference between the treated group with an injection dose of 0.2 ml/kg BW and the control group. The number of immunoreactive cells of HSD3B1 with an injection dose of 0.5 ml/kg BW was greater compared to the group that received an injection dose of 0.2 ml/kg BW. The intensity of HSD3B1 and HSD17B1 increased every week. The p450scc immunoreactive cells were only found in Leydig cells. The intensity of positive cells of p450scc in the treated group with an injection dose of 0.5 ml/kg BW was more intense, compared to the treated group with an injection dose of 0.2 ml/kg BW. The results of the current study showed that the injection of mesenchymal stem cells-conditioned medium can improve the regeneration of spermatogenic cells, and recover spermatogenesis proved by positive cells of HSD3B1, HSD17B1, and p450scc as markers of steroid production.

Keywords: Cisplatin, HSD17B1, HSD3B1, Mesenchymal stem cells-conditioned medium, P450scc, Testicular dysfunction

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Research Paper

The Influence of Basil Seed Hydroethanolic Extract on the Skin Wound Healing in Diabetic Male Rats.

Hadi M, Moghtadaei-Khorasgani E, and Etesamnia MH.

World Vet. J. 11(3): 377-383, 2021; pii:S232245682100049-11DOI: <https://dx.doi.org/10.54203/scil.2021.wvj49>

The fresh basil seeds were first washed with saline and placed in 10% formalin for 48 hours. Then, the samples were defoliated in a tissue processor with alcohol of 70, 80, 90% and absolute in each for one hour. In the next step, each sample was cleared with xylol for 48 minutes. Impregnation with methacrylate was then done for a minimum of 2 and 3 hours. In the next step, the samples were rehydrated. The sections were made with a thickness of 5 microns by microtome and the sections were placed in a container of bar water. Incubation was then performed at 45 °C in vacuum pump for 1 to 2 hours. In each step, painting was done. In each step, the first, second and third dishes are 5 minutes each. The rings were first absolute alcohol, 95, 90, 70 and distilled water, respectively, then staining hematoxylin in the next stage after washing with running water. Then impregnate with distilled acid, rinse with running water and stain with eosin dye to stain the cytoplasm. Then it goes through the steps of alcohol 70, 80, 95% and absolute alcohol and it is cleared again with xylol and then it is mounted.

ABSTRACT: Diabetic wounds and *cutaneous* wounds are important issues in medical science. Basil is an herbaceous plant and has compounds such as terpenes, flavonoids, and antioxidant properties. A total of 50 male Wistar rats were allocated into 10 groups including the healthy group without treatment, the healthy group treated with 1% phenytoin, eucerin, 5% basil seed extract, 10% basil seed, diabetic group without treatment, 1% phenytoin, eucerin. After anesthesia of rats, we made a 4 cm² wound on the back of the animal, and different histopathological characteristics were examined, and also on days 3, 7, and 21, the area of wounds was analyzed. In the healthy group treated with 10% basil seed extract, it was revealed that the wound size was significantly healed in the research days. In the diabetic rat groups, the decrease in the wound area was not significant and there was no significant difference between groups. Histopathological findings on day 21 in the healthy group treated with 10% basil seed extract revealed proper epidermis formation and relatively dense dermis containing collagen filaments. While in the diabetic groups, healing was slower. The results indicated that basil extract with anti-inflammatory and antioxidant characteristics can quicken the repair of *cutaneous* wounds.

Keywords: Basil, Diabetes, Histopathology, Wound

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Research Paper

Concurrent Respiratory Disease in Broiler Chickens in Egypt during 2020.

Yehia N, Amer F, Samir A, Samy M, Sedeek A, Rebie N, Mohammed W, and Hagag N.

World Vet. J. 11(3): 384-394, 2021; pii:S232245682100050-11DOI: <https://dx.doi.org/10.54203/scil.2021.wvj50>

Yehia N, Amer F, Samir A, Samy M, Sedeek A, Rebie N, Mohammed W, and Hagag N (2021). Concurrent Respiratory Disease in Broiler Chickens in Egypt during 2020. *World Vet. J.*, 11 (3): 384-394.

ABSTRACT: Poultry production has been affected by multiple respiratory diseases triggering serious economic losses in Egypt. The current study aimed to investigate the situation and genetic evolution of respiratory diseases in Egypt during 2020. A total of 53 samples were collected from infected flocks suffering from respiratory signs and variable mortality rates from nine governorates in Egypt during 2020. The collected samples were examined for the detection of respiratory disease viruses (Avian influenza virus (AIV (H5N8, H9N2), Infectious bronchitis virus (IBV), and Newcastle disease virus (NDV)) by rRT-PCR. The single infection was confirmed in 90.6% (37.7% I.B, 30.2% AIV (H5N8), 9.4% I.B and 5.7% NDV) and co-infection of HPAIV (H5N8) + I.BV and LPAIV (H9N2) +IBV were detected in 3.8% of nine governorates. The HA gene of HPAIV (H5N8) was cluster to clad 2.3.4.4.1b in a new branch with characteristic specific mutations especially in T140A in antigenic site A and R72S in the receptor-binding site, compared to A/duck/Egypt/F446/2017 with low A.A identity percent with vaccinal strains of H5N1 and H5N2 reaching to 91.9-94% and 84.6%, respectively. The HA gene of AIV (H9N2) belonged to A/quail/Hong Kong/G1/97-like virus clustered with group B with a specific mutation (212I) that may affect the human transmission of the virus. The HVRs of S1 gene of IBV cluster to GI23 (Egy Var I) clad with multiple mutations in HVR1 and HVR2, compared to IBV/CU/4/2014 and low identity percent (68.3-78.8%) with vaccine strains (H120, M41, 4/91). In conclusion, respiratory disease continues to circulate and rapidly evolve in Egypt during 2020.

Keywords: HPAIV (H5N8), IBV, Genetic characterization, LPAIV(H9N2), Respiratory disease

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Research Paper

Molecular Detection and Antibiotic Sensitivity of *Salmonella* Species Isolated from Goat Feces in Sylhet District of Bangladesh.

Sabur MA, Das MR, Uddin MB, Rahman MM, Islam MR, Chowdhury MSR and Hossain MM.

World Vet. J. 11(3): 395-401, 2021; pii:S232245682100051-11

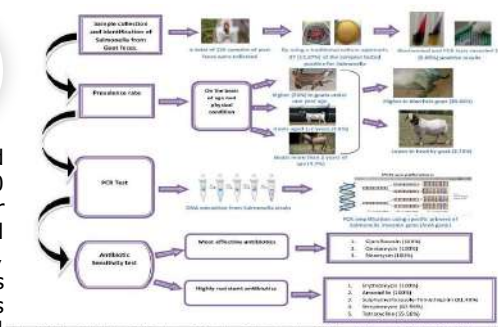
DOI: <https://dx.doi.org/10.54203/scil.2021.wvj51>



ABSTRACT: The present study aimed at the molecular detection of *Salmonella* species from feces of goats and the characterization of the isolated *Salmonella* by biochemical and antimicrobial sensitivity techniques. A total of 220 goat feces samples were collected, of which 27 (12.27%) were positive for *Salmonella* by conventional culture methods and 20 (9.09%) by biochemical and PCR techniques. The prevalence was higher in goats under one year of age (20%), compared to older animals aged one to two years (7.8%) and more than two years of age (4.7%), respectively. Moreover, the prevalence of diarrheic goats was significantly higher (38.46%) than healthy animals (2.76%). DNA was extracted from *Salmonella* strains and amplified by PCR using the specific primers of *Salmonella* invasion gene (*invA* gene). The antibiotic sensitivity test indicated that Ciprofloxacin (100 percent sensitivity), Gentamycin (100 percent sensitivity), and Neomycin (100 percent sensitivity) were the most effective antibiotics for the majority of *Salmonella* isolates. On the other hand, *Salmonella* isolates were found to have substantially high resistance to Erythromycin (100%), Amoxicillin (100%), Trimethoprim-Sulfamethoxazole (81.48%), Streptomycin (62.96%), and Tetracycline (55.56 percent). Since the rate of *Salmonella* carriers was relatively high, eating goat meat could increase the risk of foodborne salmonellosis.

Keywords: Antibiotic sensitivity, Goat isolation, PCR detection, *Salmonella*

[Full text-[PDF](#)] [[XML](#)] [[Google Scholar](#)]



Sakur MA, Das MR, Uddin MR, Bakim MM, Islam MR, Chowdhury MSR and Hossain MM (2021). Molecular Detection and Antibiotic Sensitivity of *Salmonella* Species Isolated from Goat Feces in Sylhet District of Bangladesh. World Vet. J. 11(3): 395-401.

Research Paper

Antibiotic Resistance Profile of *Escherichia coli* Isolated from Bovine Subclinical Mastitis of Dairy Farms in Algeria from 2017 to 2019.

Ghallache L, Mohamed-Cherif A, China B, Mebkhouf F, Boilattabi N, Bouchemal A, Rebia A, Ayachi A, Khelef D, Miroud K, and Ait-Oudhia Kh.

World Vet. J. 11(3): 402-415, 2021; pii:S232245682100052-11

DOI: <https://dx.doi.org/10.54203/scil.2021.wvj52>



ABSTRACT: Mastitis in cows is a major problem in dairy farms leading to a decrease in the quantity and quality of milk. The aim of the present study was to examine the association between the presence of *Escherichia coli* (*E. coli*) in milk and the subclinical mastitis, and to characterize the antibiotic resistance profiles of the isolated *E. coli*. In the current study, a total of 360 cow raw milk samples from three dairy farms of the region of Algiers were analyzed. The analysis period lasted from Spring 2017 to Winter 2019. The California Mastitis Test (CMT) was applied to detect subclinical mastitis. The *E. coli* strains were isolated from milk using conventional bacteriological methods. The antibiotic resistance profile of the isolated *E. coli* strains to 12 different antibiotics was tested using the disk diffusion method. On β -lactamase-producing strains, a double diffusion test was applied to identify the Extended-spectrum β -lactamase (ESBL) phenotype. Finally, the *ctXx-M* genes were amplified by PCR. Two-thirds (66.4%) of the milk samples were positive for the CMT test. A total of 97 *E. coli* strains were isolated from the milk samples, their resistance to antibiotics was tested, and 3.1% of the strains were resistant to trimethoprim-sulfamethoxazole, 6.2% to chloramphenicol, 12.3% to gentamicin, 13.4% to colistin, 23.3% to amoxicillin/clavulanate, 31.9% to kanamycin, 39.2% to enrofloxacin, 51.5% to cefotaxime, 52% to tetracycline, 57.7% to ampicillin, 74.3% to nalidixic acid, and 75.3% to amoxicillin. Furthermore, most of the *E. coli* strains (92.8%) were resistant to more than one antibiotic with a Multiple Antibiotic Resistance index ranging from 0 to 0.8. The 50 strains resistant to cefotaxime were analyzed for an ESBL phenotype. 39 of them (78%) were positive to the double-disk synergy test. Among the 39 ESBL positive strains, 27 (69.2%) were confirmed for the presence of a CTX-M gene by PCR. The present study showed that multiple drug-resistant *E. coli*, including ESBL-carriers, were frequently isolated from the milk of dairy cows in Algeria. The results underlined that the use of antibiotics on farms must be reasoned to avoid the spread of resistant strains in animals and human populations.

Keywords: Antibiotic Resistance, Cows, CTX-M gene, *Escherichia coli*, Milk, Subclinical Mastitis

[Full text-[PDF](#)] [[XML](#)] [[Google Scholar](#)]



Ghallache L, Mohamed-Cherif A, China B, Mebkhouf F, Boilattabi N, Bouchemal A, Rebia A, Ayachi A, Khelef D, Miroud K, and Ait-Oudhia Kh (2021). Antibiotic Resistance Profile of *Escherichia coli* Isolated from Bovine Subclinical Mastitis of Dairy Farms in Algeria from 2017 to 2019. World Vet. J. 11(3): 402-415.

Research Paper

Effect of Larvicidal Extract N-Hexane Lime Leaves (*Citrus hystrix*) on Larva Instar III Mosquito (*Culex quinquefasciatus*)

Hanif M, Lastuti NDR, and Kurnijasanti R.

World Vet. J. 11(3): 416-421, 2021; pii:S232245682100053-11

DOI: <https://dx.doi.org/10.54203/scil.2021.wvj53>



Hanif M, Lastuti NDR, and Kurnijasanti R (2021). Effect of Larvicidal Extract N-Hexane Lime Leaves (*Citrus hystrix*) on Larva Instar III Mosquito (*Culex quinquefasciatus*). World Vet. J. 11(3): 416-421.

ABSTRACT: *Culex quinquefasciatus* mosquitoes are a vector of transmission of several types of diseases, such as filariasis, Japanese encephalitis, and dirofilariasis. Larval control is the key strategy of disease control programs caused by vectors around the world because it can prevent larvae to enter the adult stage. Control of mosquito larvae that are often used is chemically controlled. *Citrus hystrix* is a natural plant and contains chemical compounds that have biological activity, such as flavonoids, carotenoids, and limonoids. The present study aimed to determine the effect of citrus leaf extract (*Citrus hystrix*) on the mortality of the larva *Culex quinquefasciatus*. The method used in the current study was a laboratory experimental study method with the experimental design using a completely randomized design. The research plot carried out was the rearing larvae of *Culex quinquefasciatus*, the manufacture of citrus leaf extract, the manufacture of larvicidal material, and the observation phase after treatment. Extract N-hexane *omplet name hystrix* leaf has high toxicity because it contains essential oils, flavonoids, alkaloids, terpenoids, saponins, and Limonoids. The Optimal concentration of N-hexane extracts of citrus leaves that lead to larval mortality of *Culex quinquefasciatus* in vitro amounted to 93.33% on 4 hours of observation with a concentration of 4000 ppm. Extract N-hexane lime leaf has a larvicidal effect on the larva *Culex quinquefasciatus* in vitro.

Keywords: *Citrus hystrix*, *Culex quinquefasciatus*, Extract, Mosquitoes, Larvicide

[Full text-[PDF](#)] [XML] [Google Scholar] [Crossref Metadata]

Research Paper

Effect of Processed Lemon Pulp with *Saccharomyces cerevisiae* in Zaribi Goats Diet on Milk Production, Nutrients Digestibility, Blood Parameters, and Reproductive Performance

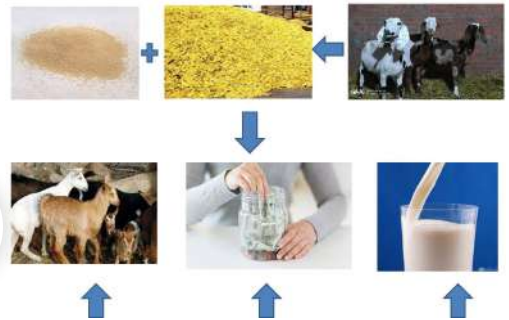
Sayed HAE, El-Maghraby MM, and Elbadawy MM.

World Vet. J. 11(3): 422-430, 2021; pii:S232245682100054-11

DOI: <https://dx.doi.org/10.54203/scil.2021.wvj54>



Sayed HAE, El-Maghraby MM, and Elbadawy MM (2021). Effect of Processed Lemon Pulp with *Saccharomyces cerevisiae* in Zaribi Goats Diet on Milk Production, Nutrients Digestibility, Blood Parameters, and Reproductive Performance. *World Vet. J.* 11 (3): 422-430.



ABSTRACT: The aim of the present study was to evaluate the effect of replacing concentrate feed (CFM) mixture with different levels of treated lemon pulp (TLP) on milk production and composition, nutrients digestibility, and blood parameters of Zaribi goats. A total of 24 Zaribi female goats at the end of the pregnant period were divided into three groups of eight according to live weight. Concentrate feed mixture and clover hay at a rate of 50:50 were offered twice a day. Treated lemon pulp replaced CFM at rates 0%, 25%, and 50 % in diets of R1, R2, and R3 treatment groups, respectively. The feeding trial lasted 90 days. Dry matter digestibility and Nitrogen free extract digestibility were significantly raised by the increased level of TLP in groups R2 (72.37% and 70.36%) and R3 (72.28% and 70.30%), compared to (70.99% and 68.51%) in R1, respectively. The same trend was observed for organic matter digestibility (OMD), crude protein digestibility (CPD), crude fiber digestibility (CFD), and ether extract digestibility (EED) with R3, compared with either R1 or R2. However, there were insignificant differences between R1 and R2 in terms of OMD, CPD, and EED but CFD was significantly higher in R2, compared to R1. There was a significant increase in the milk yield by the increased level of TLP in the diet and for R2 (1448.4g/h/d) and R3(1558.7g/h/d), while it was reported as 1377.6 g/h/d in the control group. Feeding dams on the R3 diet had a significant effect on improving total antioxidant capacity by 63.5%, compared with control. The results of the present study indicated that the replacement of CFM by TLP decreased the costs of feed and increase milk production for the replacement level by 50% (R3). Moreover, no adverse effects were noticed on nutrients digestibility and blood parameters of the investigated samples.

Keywords: Blood parameters, Lemon pulp, Milk production, *Saccharomyces cerevisiae*

[Full text-[PDF](#)] [XML] [Google Scholar]

Short Communication

The Effects of Different Concentrations of Bay Leaf Extract on Shelf Life of Preserved Scad Fish

Fuaidah P, Triastuti J, and Pramono H.

World Vet. J. 11(3): 431-438, 2021; pii:S232245682100055-11

DOI: <https://dx.doi.org/10.54203/scil.2021.wvj55>



Fuaidah P, Triastuti J, and Pramono H (2021). The Effects of Different Concentrations of Bay Leaf Extract on Shelf Life of Preserved Scad Fish. *World Vet. J.* 11 (3): 431-438.

ABSTRACT: Scad fish (*Decapterus kurroides*) is the most productive fish species. To provide the added value, fishermen can process scad fish into various forms of processed products, such as preserved fish, which extend the shelf life. In order to extend the shelf life of canned fish, natural preservatives could be used that contain antimicrobial agents, such as bay leaves. The present study aimed to determine the effects of bay leaf extract in different concentrations on the shelf life of preserved scad fish. In the present study, the completely randomized design was used as an experimental research method. The treatments given differed in terms of the concentration of the bay leaf extract. The groups were treated as the scad fish without bay leaf extract (A), scad fish soaked in 6% of bay leaf extract (B), scad fish soaked in 7% of bay leaf extract (C), scad fish soaked in 8% of bay leaf extract (D), and scad fish soaked in 9% of bay leaf extract (E). The analyzed parameters included the total plate count, pH, and water level test using Analysis of Variance (ANOVA). The supportive parameters observed included an organoleptic test. The results indicated that the lowest total bacterial count from the beginning to the end of the experiment (18 hours) occurred on treatment C (7% of bay leaf extract addition), which was 1.54×10^3 to 5.85×10^6 . Regarding the water level test from the beginning to the end of the experiment, treatment A (control) was not significantly different from other treatments. The difference in the concentration of bay leaf extract on scad fish effectively inhibited the growth of bacterial colonies. In conclusion,

adding bay leaf extract to scad fish could inhibit bacteria for up to 12 hours. Treatment C (7% of bay leaf extract) gave the best results since this concentration level could inhibit the bacteria on scad fish.

Keywords: Bay leaf, Preservation, Scad fish, Shelf life

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Research Paper

Effects of Laying Cycle Periods on Egg Quality, Egg Chemical Composition, and Reproductive Performance of Japanese Quail Breeders Reared in Northern Algeria

Berrama Z, Souames S, Merati R, Korteby HM, Chirane MS, Negab N, Hettab K, Idris H, Morzouglal N, and Temim S.

World Vet. J. 11(3): 439-447, 2021; pii:S232245682100056-11

DOI: <https://dx.doi.org/10.54203/scil.2021.wvj56>

ABSTRACT: Egg quality traits in quail breeders depend on various factors which may influence embryo survival during incubation, affecting the chicks' production and quality. The current study aimed to determine the effect of the laying period on the external and internal quality of the egg, the chemical composition of albumen, yolk, and eggshell as well as the reproductive parameters of quail breeders. A total of 450 quails (*Coturnix japonica*) obtained from the same hatchery, were reared in a battery cage with a sex ratio of 1 male to 3 females. All the quails were subjected to standard breeding conditions and fed a balanced laying diet. A total of 960 eggs were collected at two different periods of the laying cycle, the beginning period of the laying (BP indicating 10 weeks of quail age) and the peak period of laying (PP showing 20 weeks of quail age). At each laying period, 30 eggs were used to analyze the various parameters of egg quality, and 450 eggs were randomly selected to assess the reproductive performances. Overall, no differences in the external quality of eggs, such as egg length, egg width, and the egg shape index, were recorded between the two laying periods. Apart from albumen weight that tended to be higher at the peak laying period, the shell and the yolk weights were not affected by the period of laying. Additionally, Japanese quail tend to deposit similar proportions of shell, albumen, and yolk at the two periods of laying. Likewise, the rate of dry matter of the three egg components, shell mineral concentrations, and yolk fat concentrations did not show any noticeable variation with the laying period. The most significant effect of the laying period was related to the potential reduction in the total protein content of the albumen and the yolk of eggs laid at the peak period of laying. Finally, the laying period did not significantly affect the fertility and hatchability rate of the incubated quail eggs but slightly improved the embryonic mortality rate during the peak laying phase.

Keywords: Albumen, *Coturnix japonica*, Eggshell, Fertility, Quail

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Research Paper

The Role of Salinity in Histopathology Description of Jatim Bulan Tilapia Juvenile (*Oreochromis niloticus*) Exposed by Lead (PB)

Batista FR, Triastuti J, and Pursetyo KT.

World Vet. J. 11(3): 448-455, 2021; pii:S232245682100057-11

DOI: <https://dx.doi.org/10.54203/scil.2021.wvj57>

ABSTRACT: The decrease in the number of lands for aquaculture will cause the freshwater fish aquaculture, especially tilapia is reared. As one of the efforts to survive, the breeders use the coastal area to anticipate, so the freshwater fish can adapt to the sea waters. Heavy metal pollution near the coasts (or in the coastal waters) has great potential impacts on the environment. The present study aimed to find out the effect of Lead (Pb, heavy-metal) exposure on the Jatimbulan juvenile tilapia with the different salinity towards histopathology description of the gill, intestine, and the skin tissues. The method used was an experimental method with a completely randomized design using six treatments and three times repetition. The observed parameters included the changes in histopathology description of gill, intestine, and skin organs of Jatimbulan juvenile tilapia. Kruskal-Wallis scoring method was used for data analysis and was continued by Mann-Whitney. The result of the current study showed the effect of salinity towards histopathology description of gill, intestine, and skin of Jatimbulan juvenile tilapia. The effect of changes was proven with the histopathology description in the form of the damage of edema, hyperplasia, and necrosis on the gill tissue; the damage of edema, atrophy, and necrosis on the gill tissue as well as the damage of edema, atrophy, hemorrhagic and necrosis on the gill tissue. From the result of the current study which has been conducted, it can be concluded that the heavy-metal exposure by lead was 0.03 ppm on the salinity of 10 ppt and 20 ppt. given the significant effect on the histopathology description of gill, intestine, and skin of Jatimbulan juvenile tilapia.

Keywords: Histopathology, Lead, Metal, *Oreochromis niloticus*, Salinity

[Full text-[PDF](#)] [XML] [Google Scholar]



Berrama Z, Souames S, Merati R, Korteby HM, Chirane MS, Negab N, Hettab K, Idris H, Morzouglal N and Temim S (2021) Effects of Laying Cycle Periods on Egg Quality, Egg Chemical Composition, and Reproductive Performance of Japanese Quail Breeders Reared in Northern Algeria *World Vet. J.*, 11 (3): 439-447. <https://www.wvj.science-line.com/>

Research Paper

The Role of Salinity in Histopathology Description of Jatim Bulan Tilapia Juvenile (*Oreochromis niloticus*) Exposed by Lead (PB)

Batista FR, Triastuti J, and Pursetyo KT.

World Vet. J. 11(3): 448-455, 2021; pii:S232245682100057-11

DOI: <https://dx.doi.org/10.54203/scil.2021.wvj57>



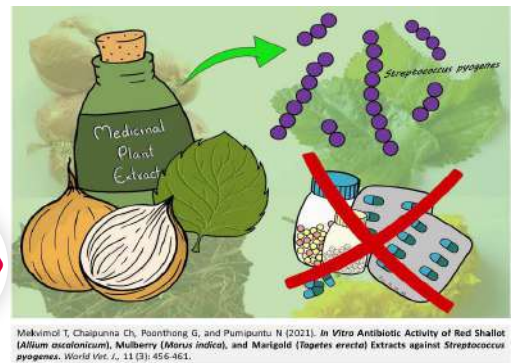
Histopathology Description of Jatim Bulan Tilapia Juvenile

Research Paper

In Vitro Antibiotic Activity of red Shallot (*Allium ascalonicum*), Mulberry (*Morus indica*), and Marigold (*Tagetes erecta*) Extracts against *Streptococcus pyogenes*

Mekvimol T, Chaipunna Ch, Poonthong G, and Pumipuntu N.

World Vet. J. 11(3): 456-461, 2021; pii:S232245682100058-11

DOI: <https://dx.doi.org/10.54203/scil.2021.wvj58>

Mekvimol T, Chaipunna Ch, Poonthong G, and Pumipuntu N (2021). In Vitro Antibiotic Activity of Red Shallot (*Allium ascalonicum*), Mulberry (*Morus indica*), and Marigold (*Tagetes erecta*) Extracts against *Streptococcus pyogenes*. World Vet. J., 11 (3): 456-461.

ABSTRACT: Bacterial infection is a major global health concern. One of the critical problems is the widespread of antimicrobial-resistant bacteria from inappropriate and prolonged use of antimicrobial agents in both humans and animals. Plant extracts might afford the chance to replace antibiotic drugs and reduce the emerging of antimicrobial-resistant bacteria. This study aimed to examine the antibiotic activity of ethanolic crude extracts of some Thai medicinal plants grouping in their parts as whole onions of red shallot (*Allium ascalonicum*), petals of marigold (*Tagetes erecta*), mulberry leaves, and root barks (*Morus indica*) to inhibit the growth of *Streptococcus pyogenes*. The antibiotic activities of the crude extract of three Thai medicinal plants using absolute ethanol were trialed against *Streptococcus pyogenes* using the disk diffusion method. Erythromycin and Ceftriaxone discs were chosen to be positive control standards as the representative of antibiotic drugs. Each dried plant extracts was prepared to test the inhibition with a concentration at 25, 50, and 75 mg/mL stock solution. The results showed that three groups from all testing groups of Thai medicinal plant extracts had the potential of antibiotic activity against *S. pyogenes*. The highest antibiotic activity against *S. pyogenes* was detected from whole onion extract red shallot followed by the extract of the mulberry leaves and root barks of mulberry strain Nakhon Ratchasima 60 (Nak 60) while the extract of marigold petal did not present antibiotic activity. The results revealed that crude extract of those two Thai medicinal plants, including red shallot and mulberry, had antibiotic activity against bacterial growth of *S. pyogenes* in the experiment and these medical Thai plants had potential benefits for developing as alternative treatment agents for *S. pyogenes* infections in both humans and animals in the future.

Keywords: Antibiotic activity, Ethanolic crude extract, Thai medicinal plants, *Streptococcus pyogenes*

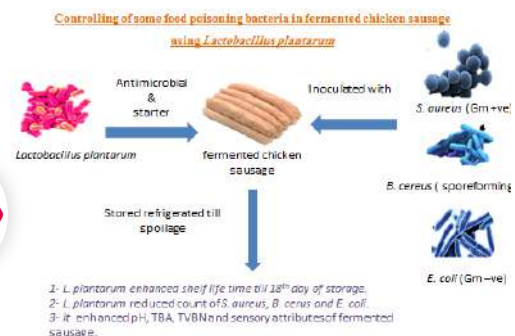
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Research Paper

Controlling Food Poisoning Bacteria in Fermented Chicken Sausage Using *Lactobacillus plantarum*

Elsabagh R, Nada ShM, and Abd-Elaaty EM.

World Vet. J. 11(3): 462-468, 2021; pii:S232245682100059-11

DOI: <https://dx.doi.org/10.54203/scil.2021.wvj59>

ABSTRACT: *Lactobacillus plantarum* (*L. plantarum*) bacteria is generally recognized as safe and widely used in the food industry. The current study aimed to study the antimicrobial effects of *L. plantarum* against some food poisoning microorganisms, such as *Staphylococcus aureus* (*S. aureus*), *Bacillus cereus* (*B. cereus*), and *Escherichia coli* (*E. coli*) in oriental fermented chicken sausage for 18 days of storage at 4°C. The *L. plantarum* has broad-spectrum antimicrobial effects that enhance the quality and safety of food products. *L. plantarum* reduced the count of *S. aureus*, *B. cereus*, and *E. coli* to 1.54, 4.26, and 3.03 Log₁₀, respectively, after 18 days of refrigerated storage. Moreover, there were significant effects of *L. plantarum* on pH, thiobarbituric acid, total volatile basic nitrogen, and sensory attributes of fermented sausage samples during storage time. It was revealed that *L. plantarum* enhanced the physico-chemical, sensory attributes, and shelf life of fermented chicken sausage. Moreover, *L. plantarum* inhibited the inoculated food poisoning bacteria in fermented chicken sausage. In conclusion, it is recommended to use *L. plantarum* in fermented meat products as a starter and a bio-preservative to enhance the quality of the fermented chicken sausage.

Keywords: Chicken sausage, Food safety, *Lactobacillus plantarum*, Probiotic

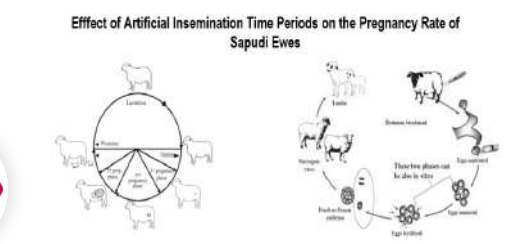
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Short Communication

The Effect of the Different Artificial Insemination Time Periods on the Pregnancy Rate of Sapudi Ewes

Putri CD, Ismudiono, and Poetranto ED.

World Vet. J. 11(3): 469-473, 2021; pii:S232245682100060-11

DOI: <https://dx.doi.org/10.54203/scil.2021.wvj60>

ABSTRACT: Brucellosis Artificial insemination is required to increase the reproduction rate in ruminant breeding. The artificial insemination success rate in sheep only reaches 47.6%, whereas the proposed ideal rate is 70%. One of the factors influencing the artificial insemination success rates in sheep is improper estrus detection, resulting in no fertilization. The present study aimed to determine the effect of different artificial insemination time periods on the pregnancy rates of Sapudi ewes. The research design was based on a completely randomized design. A total of 20 female Sapudi sheep were divided into four treatment groups with five repetitions (for each group). In addition, the observed variables were artificial insemination time in Sapudi ewes. Estrus synchronization in ewes was conducted by injecting PGF2 α . The results of the research indicated that ewes subjected to artificial insemination 6, 12, 18, and 24 hours after estrus had a pregnancy rate of 20%, 100%, 60%, and, 60%, respectively. It can be concluded that the time differences in artificial insemination significantly influence the pregnancy rate in Sapudi ewes'.

Keywords: Artificial insemination, Estrus, Pregnancy rate, Sapudi ewes

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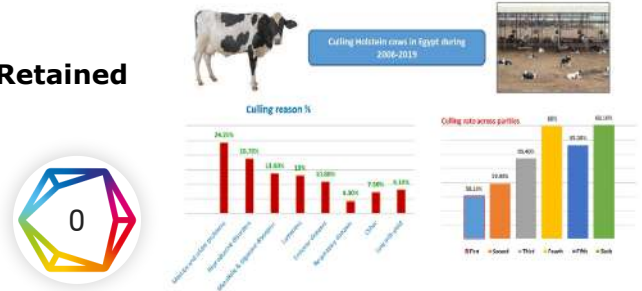
Research Paper

Milk Production and Reproductive Performance of Retained and Culled Cows in a Large Holstein Herd in Egypt

Fahim NH, Ibrahim MA-AM, Amin AH, and Sadek RR.

World Vet. J. 11(3): 474-483, 2021; pii:S232245682100061-11

DOI: <https://dx.doi.org/10.54203/scil.2021.wvj61>



ABSTRACT: The study aimed to identify the culling reasons of Holstein cows raised in a large commercial herd in Egypt with emphasis on the performance of retained and culled cows. A total of 31534 complete lactation records for 10994 cows calved from 2008 to 2019 were used. The overall rate of culling per lactation was 61.1%. Involuntary culling represented 92% of all culling cases. The reasons for culling included mastitis and udder problems (24.2%), reproductive disorders (18.7%), metabolic and digestive disorders (13.6%), lameness (13%), endemic diseases (10.8%), low milk yield (8.1%), respiratory diseases (4.3%) and unknown causes (7.3%). Means of 305-day milk yield and daily milk yield were significantly lower in culled cows than the retained ones. On the other hand, no significant differences were observed between culled and retained cows for days open and the number of services/conception. The high involuntary culling rate of Holstein under the Egyptian conditions revealed that management practices regarding mastitis prevention and reproductive efficiency should be improved.

Keywords: Culling reasons, Egypt, Holstein, Milk production, Reproductive performance

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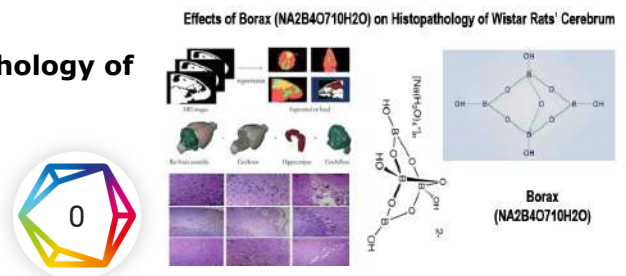
Short Communication

The Effects of Borax ($\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$) on Histopathology of Wistar Rats' Cerebrum (*Rattus norvegicus*)

Elfan F, Kuncorojakti S, and Triakoso N.

World Vet. J. 11(3): 484-488, 2021; pii:S232245682100062-11

DOI: <https://dx.doi.org/10.54203/scil.2021.wvj62>



ABSTRACT: The present study aimed to determine the effects of borax ($\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$) addition on the changes of histological cerebrum imaging in the brains of white mice (*Rattus norvegicus*). The current research was an experimental study with randomization of 24 white mice that were divided into four treatment groups with five repetitions. Borax was dissolved for each treatment with a dose of 19 mg/mouse/day, 26 mg/mouse/day, and 37 mg/mouse/day, and it was administered orally for 14 days. Then, it was analyzed statistically using the Kruskal-Wallis test. The statistical analysis results suggested that there were significantly different results in each treatment group. The control treatment with an administration dose of 26 mg/rat/day had a significantly different result in the worst cloudy swelling degeneration of cerebrum in histopathology imaging on Wistar rats (*Rattus norvegicus*). Using the Mann-Whitney test, it was found that the dose of borax at 37 mg/rat/day led to significant difference, compared to the other treatment groups, which means that 37 mg/rat/day of borax caused the worst pyramidal cell necrosis in histopathology imaging of the cerebrum on white mice. Borax exposure on Wistar rats (*Rattus norvegicus*) can cause cloudy swelling at a dose of 26mg/head/day, and pyramidal cell necrosis at a dose of 37 mg/head/day.

Keywords: Borax, Cerebrum, Cloudy swelling, Necrosis

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Research Paper

Evaluation of Milk Yield and Reproductive Performance of Pure Holstein and Its F1 Crossbreds with Montbeliarde in Egypt

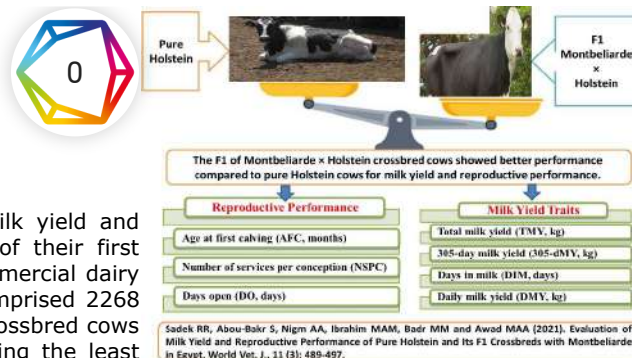
Sadek RR, Abou-Bakr S, Nigm AA, Ibrahim MAM, Badr MM and Awad MAA.

World Vet. J. 11(3): 489-497, 2021; pii:S232245682100063-11

DOI: <https://dx.doi.org/10.54203/scil.2021.wvj63>

ABSTRACT: The present study was carried out to compare the milk yield and reproductive performance of pure Holstein (HO) cows with those of their first generation (F1) crossbreds with Montbeliarde cows (MO) in four commercial dairy herds under Egyptian conditions. Data used in the current study comprised 2268 records for the first four lactations of 531 HO and 536 MO × HO F1 crossbred cows during the period between 2012 and 2020. Data were analyzed using the least squares method by XLSTAT software. The MO × HO crossbred cows were significantly superior compared with pure HO cows for 305-day milk yield, scoring 9210 ± 96 kg versus 7987 ± 149 kg. Moreover, MO × HO F1 crossbred cows had a significantly higher daily milk yield (30.0 ± 0.45 kg) than pure HO cows (25.9 ± 0.52 kg). However, pure HO cows had significantly greater days in milk (399 ± 6 days) than MO × HO crossbred cows (341 ± 5.2 days). With regard to reproductive performance, MO × HO F1 crossbred cows had significantly less number of services per conception and days open than pure HO cows (2.6 ± 0.16 vs. 3.7 ± 0.18) and (132 ± 5.2 days vs. 190 ± 6 days), respectively. However, the statistical difference between MO × HO F1 crossbred cows and pure HO cows for age at first calving was not significant (22.9 ± 0.11 vs. 23.1 ± 0.15 months, respectively). It can be concluded that under Egyptian subtropical conditions, the first generation of MO × HO crossbred cows exhibit better performance, compared to pure HO cows in milk yield and reproductive traits. These findings could provide an effective strategic option for the genetic improvement of dairy cattle in hot subtropical regions.

Keywords: Crossbreeding, Egypt, Holstein, Milk Yield, Montbeliarde, Reproduction



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Short Communication

Intensity and Predilection of Helminth Parasites of the Red Snapper (*Lutjanus argentimaculatus*)

Prasetya AW, Kismiyati, and Subekti S.

World Vet. J. 11(3): 498-503, 2021; pii:S232245682100064-11

DOI: <https://dx.doi.org/10.54203/scil.2021.wvj64>

ABSTRACT: Marine fish, particularly the red snappers, are often exposed to helminth parasitic infestation. As a result of the parasitic infestation, the fish population, the fish weight, and the morphological changes in the fish are shrinking. The present research aimed to find out the intensity and predilection of the helminth ectoparasites over the infection of the red snapper (*Lutjanus argentimaculatus*) and employed the survey method for this purpose. The sampling was carried out by the purposive sampling technique. The sample obtained consisted of 30 fish, 20% of the total red snapper population of 150 fish reared in the floating net cages of Balai Besar Perikanan Budidaya Laut Lampung. The intensity of the fish infestation by a mixture of *Haliotrema epinepheli* and *Benedenia epinepheli* was 132.5 individuals/fish. *Neobenedenia girellae* and *Haliotrema epinepheli* infected fish with an intensity of 149.41 individuals/fish. The 66.7% of *Benedenia epinepheli* had a predilection for the dorsal fin, and 33.3% for the anal fin. In *Neobenedenia girellae*, 57.1% had a predilection for the body surface, 37.2% for the head surface, and 5.7% for the dorsal fin. In conclusion, all sampled fish were positively infected with helminth ectoparasites, including *Neobenedenia girellae*, *Haliotrema epinepheli*, and *Benedenia epinepheli*.

Keywords: Ectoparasite, Helminths, Infestation, Red snapper

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Research Paper

Cross Reaction of *Haemonchus contortus* Protein with *Toxocara vitulorum* Anti-L2 Serum Using Western Blot Technique

Asmorowati RW, Kusnoto, and Eliyani H.

World Vet. J. 11(2): 504-509, 2021; pii:S232245682100065-11

DOI: <https://dx.doi.org/10.54203/scil.2021.wvj65>

ABSTRACT: In the adult stage, *Haemonchus contortus* worms infect the abomasum host causing anemia and even death in animals. However, identifying the *H. contortus* protein can be used as a reference for the diagnosis of diseases. The diagnosis is performed by serological cross-reaction between *H. contortus* protein and anti-L2 *Toxocara vitulorum* (*T. vitulorum*) serum using the western blot technique. The main purpose of the current research was to identify the cross-reaction between *H. contortus* proteins and anti-L2 *T. vitulorum* serum using the western blot technique. *T. vitulorum* worms were collected from the intestine of cattle and *H. contortus* worms were collected from the abomasum of goats. The first step was making antibodies by oral



infection of rats with infective eggs (L2) of *T. vitulorum*. The blood was taken 21 days after infection. Then, the blood was centrifuged at 1500 rpm for 10 minutes to get the serum. The second step was making homogenates from the whole worm extract of *H. contortus*. After crushing the worms, it was centrifuged at 5000 rpm for 15 minutes and the supernatant was taken. The supernatant was then analyzed using Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) with coomassie brilliant blue staining. The third step was the analysis of *H. contortus* protein with serum anti-L2 *T. vitulorum* using the western blot technique. From the *H. contortus* homogenates analysis using SDS-PAGE, 16 protein bands were obtained. The cross-reactions were 141.3, 81.3, 64.6, 51.3, 46.8, and 38 kDa. The data from cross-reactions suggested that the *H. contortus* protein cannot be used as a diagnostic material. It is serologically Haemonchosis because it caused false positives with diagnostic Toxocariasis.

Keywords: Cross reaction, *Haemonchus contortus*, SDS-PAGE, *Toxocara vitulorum*, Western blot

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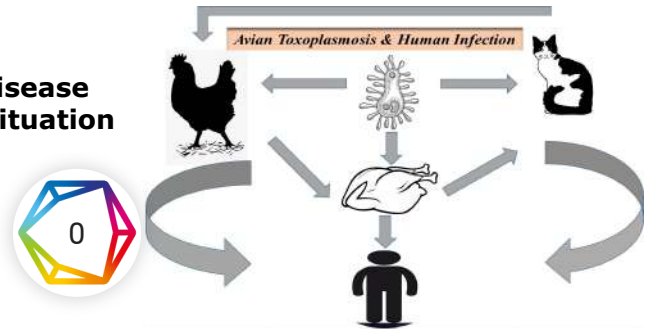
Review

A Highlight on Avian Toxoplasmosis: One Health Disease with a Special Reference to the Current Egyptian Situation

Abd El-Ghany WA.

World Vet. J. 11(2): 510-520, 2021; pii:S232245682100066-11

DOI: <https://dx.doi.org/10.54203/scil.2021.wvj66>



Abd El-Ghany WA (2021). A Highlight on Avian Toxoplasmosis: One Health Disease with a Special Reference to the Current Egyptian Situation. *World Vet. J.* 11 (3): 510-520.

ABSTRACT: This review article was developed to the infection of avian species with *Toxoplasma gondii* (*T. gondii*), diagnosis, pet bird and human infection, and control methods with a special reference to the current status of infection among the Egyptian poultry farms and population. Toxoplasmosis is a zoonotic disease caused by a unicellular, protozoan parasite *T. gondii*. Different domesticated and wild animals, as well as birds can harbor *T. gondii* and may be a potential source of infection to humans. Avian species could be infected with *T. gondii* through the ingestion of contaminated food, soil, and water with oocysts shed in the excreta of infected animals, especially cats. Poor sanitation and hygienic conditions increase the risk of infection. Consumption of food or water, as well as undercooked poultry meat or meat products containing the oocysts of the parasite, are the main sources of human infection with *T. gondii*. Diagnosis of *T. gondii* in the infected host depends on the serological detection of specific antibodies and molecular detection of the parasite. Microscopic demonstration of the oocysts and other developmental stages of the parasite in the intestine, liver, brain, and skeletal muscles tissues is another means for rapid diagnosis. Generally, a high prevalence of the disease is also reported in pet birds. Toxoplasmosis in humans is associated with abortion, congenital disorders, stillbirth, and other complications, especially in immunocompromised patients. Application of hygienic measures, as well as public awareness, are essential for the prevention and control of toxoplasmosis. In different Egyptian governorates, a high prevalence of *T. gondii* has been detected in animals, birds, and humans. High incidence of infection was recorded due to the contact with *Toxoplasma* oocysts shed mainly from infected cats or other carriers. Egyptian chicken and turkey flocks and backyard birds revealed the presence of different developmental stages of the parasite and even its antibodies. In addition, human populations showed signs of toxoplasmosis with severe complications.

Keywords: Bird, Egypt, Human, *Toxoplasma gondii*, Zoonosis

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Effect of Larvicidal Extract N-Hexane Lime Leaves (*Citrus hystrix*) on Larva Instar III Mosquito (*Culex quinquefasciatus*)

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ABSTRACT

Culex quinquefasciatus mosquitoes are a vector of transmission of several types of diseases, such as filariasis, Japanese encephalitis, and dirofilariasis. Larval control is the key strategy of disease control programs caused by vectors around the world because it can prevent larvae to enter the adult stage. Control of mosquito larvae that are often used is chemically controlled. *Citrus hystrix* is a natural plant and contains chemical compounds that have biological activity, such as flavonoids, carotenoids, and limonoids. The present study aimed to determine the effect of citrus leaf extract (*Citrus hystrix*) on the mortality of the larva *Culex quinquefasciatus*. The method used in the current study was a laboratory experimental study method with the experimental design using a completely randomized design. The research plot carried out was the rearing larvae of *Culex quinquefasciatus*, the manufacture of citrus leaf extract, the manufacture of larvicidal material, and the observation phase after treatment. Extract N-hexane *omplet name hystrix* leaf has high toxicity because it contains essential oils, flavonoids, alkaloids, terpenoids, saponins, and Limonoids. The Optimal concentration of N-hexane extracts of citrus leaves that lead to larval mortality of *Culex quinquefasciatus* in vitro amounted to 93.33% on 4 hours of observation with a concentration of 4000 ppm. Extract N-hexane lime leaf has a larvicidal effect on the larva *Culex quinquefasciatus* in vitro.

Keywords: *Citrus hystrix*, *Culex quinquefasciatus*, Extract, Mosquitoes, Larvicide

INTRODUCTION

Indonesia is a tropical country that has optimal humidity and temperature so as to support insect survival. Mosquitoes are the type of insects that coexist with humans but can harm humans because of their role as destructive organisms and disease vectors (vector-borne diseases; Sumantri and Satar, 2015).

Culex (Cx.) quinquefasciatus mosquitoes are the vector of transmission of several types of diseases such as filariasis, Japanese encephalitis, and dirofilariasis (Hadi and Koesharto, 2006). Filariasis is an infectious disease caused by microfilarial worms. This disease can cause disability, psychosocial, and decreased patients' productivity. The number of filariasis cases in Indonesia increases annually. Japanese Encephalitis (JE) is a viral disease that causes encephalitis and is a problem of public health in Asia including in Indonesia. Mosquito control can be done at the larvae stage, but in the adult stage that mosquitoes can fly they can transmit the disease widely (Aradilla, 2009). The control of larvae is the key strategy of vector-borne disease control programs caused by vectors around the world because it can prevent larvae to become adults. The larvae stadium has the longest lifespan of about 10-15 days so that the exposure is also longer, adult stage or adult mosquitoes can also fly so that it can spread the disease widely. The use of insecticide as larvicide can be the most commonly used way for people to control the growth of the vector (Manimegalai and Sukanya, 2014). Control of mosquito larva that has been often used is chemical control, namely by using synthetic chemicals. It can suppress vector populations rapidly. However, control in this way when done repeatedly is less effective because it can cause resistance to larvae, death for animal predatory predators and environmental pollution (Yasmin and Fitri, 2013). Biolarvasida of a plant (vegetable) is considered quite potential replacing synthetic insecticide. Plant insecticides are generally more selective than chemical insecticides because they are directly targeted at the target insect species, but also do not pollute the environment because it is easily degraded by nature and its use is quite safe (Aktar et al., 2009). More than 2400 plant types that belong to 255 families are reported to contain pesticide ingredients. Basil, germanium,

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and zodia belong to a group of mosquito repellent plants due to their distinctive aroma. The plant contains compounds, such as methyl eugenol and some microelements, such as Linalool, Terpinol, Eugenol, Sincol, and Geraniol. In addition, there are other types of mosquito repellent including lavender, zodia, rosemary, fragrant lemongrass, and citrus kaffir lime (Kardinan, 2005). *C. hystrix* is a plant that is found easily by people. Plants derived from this genus of *Citrus* have chemical compounds that have biological activities, such as flavonoids, carotenoids, and Limonoids (Nurhaifah and Sukei, 2015). Limonoid compounds are the teranoriterpen found in the leaves of Citrus lime which is potentially antifeedant to insects, growth regulator, and toxic substances in rice lice, larvacide, antimicrobial, insect repellent, and reproductive inhibitors (Li, 2001). The limonoid compound is an analog of juvenile hormones on insects that serve as a regulator of the growth of larval cuticles (Tunaz and Uygun, 2004). Saponin is also able to become biolarvasida. Saponin compounds in extracts that are eaten by the larvae of *Cx. quinquefasciatus* may irritate the digestive tract of *Cx. Quinquefasciatus*, and damage the cell membrane of the larva. Limonoid, which is an essential oil in orange, can lead to the loss of coordination of the nerve organ of the larva *Cx. Quinquefasciatus* (Pelah et al., 2002). In addition, limonoid has the ability to inhibit the formation of cancer cells, reducing the formation of low-density lipoproteins (LDL) as well as being able to suppress the appetite from insects (Murthy et al., 2021). Therefore, this research is done to find out the benefits of citrus leaves that are thought to have effects on larvae of *Cx. quinquefasciatus*.

MATERIALS AND METHODS

The research was conducted in the entomology laboratory of The Institute of Tropical Disease (ITD), Airlangga University, and the Department of Medicine of Veterinary Faculty of Universitas Airlangga, Surabaya, Indonesia. The material used consists of 420 larvae instar III *Cx. quinquefasciatus* obtained from the laboratory of ITD. Each treatment group used a sample of 20 larvae.

The current study utilized experimental research methods of laboratories with the experimental design using a completely randomized design using simple random sampling. The study was conducted in several phases, namely the rearing phase of the larva of *Cx. quinquefasciatus*, the manufacturing stage of citrus leaf extract of *C. hystrix*, the manufacturing stage of larvacidal material, and the observation phase after treatment. In rearing the larvae of *Cx. quinquefasciatus*, selection was based on the body length (5-6 millimeters, determined by a compound microscope with calibrated eyepiece graticule) and the day after the eggs hatch (four to six days). There was a triangular thickening consisting of many comb-shaped scales, had complete its morphological structure, and clearly, the body could be divided into a part of the head, chest, and abdomen. Prior to the study, the larvae were restored in advance in order to prove that the administration proved optimal. Subsequently, 420 larvae of instar III of *Cx. quinquefasciatus* were divided into seven treatment groups, namely the control group (+) consisting of Temephos 1 ppm + aquadest AD 100 ml solution, the control group (-) consisting of a solution of aquadest 100 ml, treatment 1 entailing 25 mg of citrus leaf extract of kaffir lime + aquadest AD 100 ml, treatment 2 consisting of 50 mg of kaffir lime citrus leaf extract + aquadest AD 100 ml, treatment 3 having 100 mg Citrus leaf extract of kaffir lime + aquadest AD 100 ml, treatment 4 including 200 mg citrus leaf extracts of kaffir lime + aquadest AD 100 ml, treatment 5 consisting of 400 mg of citrus leaf extracts of kaffir lime + aquadest AD 100 ml. The larvacide extract of the kaffir lime citrus leaves was then poured into plastic cups. Then, into the plastic cups that have been contained treatment solution inserted larvae of 20 tails of each glass. Observations were performed every 4 hours for 24 hours to see the dead larvae.

The physical factors measured in the room used during the study included the pH of water, water temperature, and room temperature. Measured water pH ranges from 7.0 to 7.2. The condition is a neutral pH and qualifies for the life of larvae in water. The measured room temperature is 25°C, which is the temperature suitable for larvae growth.

RESULTS

Result of soaking larvae Instar III of *Cx. quinquefasciatus* in various concentrations of treatment for 24 hours obtained the average value and standard deviation of the number of Instar larva III *Cx. quinquefasciatus* every 4 hours observation that can be seen in Table 1.

Based on Table 1 above, it can be noted that there is a significant difference in each treatment of the percentage of mortality of Instar-III Larva ($p \leq 0.05$). However, the results of a comparison of immersion treatment in solution Temephos with extracts N-hexane citrus leaves of 4000 ppm concentrations do not show a noticeable difference. It is different from the immersion comparison of Temephos solution 1 ppm with the N-hexane extracts of citrus leaves with the concentrations of 2000, 1000, 500, and 250 ppm which show noticeable different results. Extract N-hexane citrus leaves of 4000 ppm concentrations at a 4-hour observation have shown an average death of as much as 19 tails so that it can be known that the value (LC_{90}) of extract n-hexane lime leaf is 4000 ppm. More details can be seen in Figure 1.

Based on Figure 1 of the comparative mortality ratio of Larva *Cx. quinquefasciatus* on the observation every 4 hours for 24 hours obtained the result that the relationship between the concentration of N-hexane extracts of citrus

leaves with larvae instar-larva III *Cx. quinquefasciatus* have a positive direction, meaning the higher concentrations of citrus leaf extract kaffir lime, the higher the power of the larva of mosquitoes *Cx. quinquefasciatus*. The increased immersion time at the concentrations of P1, P2, P3, and P4 showed an increase in the number of deaths each time of observation, it showed the time of immersion of N-Hexsan extract material affecting the death process of the mosquito larva *Cx. quinquefasciatus*. In negative control does not encounter the death of larvae it indicates there is no reaction of the use of aqueous solutions.

The results in the experiment indicated a significant difference in each treatment. This means that the death of the larvae of instar III *Cx. quinquefasciatus* was due to the immersion of the larvae in the kaffir lime citrus leaf extract. Insignificant differences in the positive control and the highest concentration of citrus leaf extract kaffir lime resulted from the equation of the effectiveness of citrus leaf extract in the killing of larvae.

Table 1. The results of soaking larvae every four hours

Mortality (%) Error! Bookmark not defined. Groups	Average ± standard deviation					
	4 hours	8 hours	12 hours	16 hours	20 hours	24 hours
K (+)	88.33 ± 2.88	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
K (-)	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
P1	11 ± 5.77	21.66 ± 5.77	25 ± 5	30 ± 5	38.33 ± 7.63	41.66 ± 7.63
P2	15 ± 5	28.33 ± 7.63	36.66 ± 5.77	38.33 ± 7.63	48.33 ± 10.40	51.66 ± 7.63
P3	30 ± 13.22	45 ± 13.22	56.66 ± 14.43	66.66 ± 10.40	76.66 ± 5.77	78.33 ± 7.63
P4	55 ± 5	63.33 ± 7.63	68.33 ± 10.40	73.33 ± 7.63	80 ± 10	91.66 ± 10.4
P5	93.33 ± 2.88	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0

K+: Temephos, K-: Aquadest, P1: 250 ppm, P2: 500 ppm, P3: 1000 ppm, P4: 2000 PPM, and P5: 4000 ppm

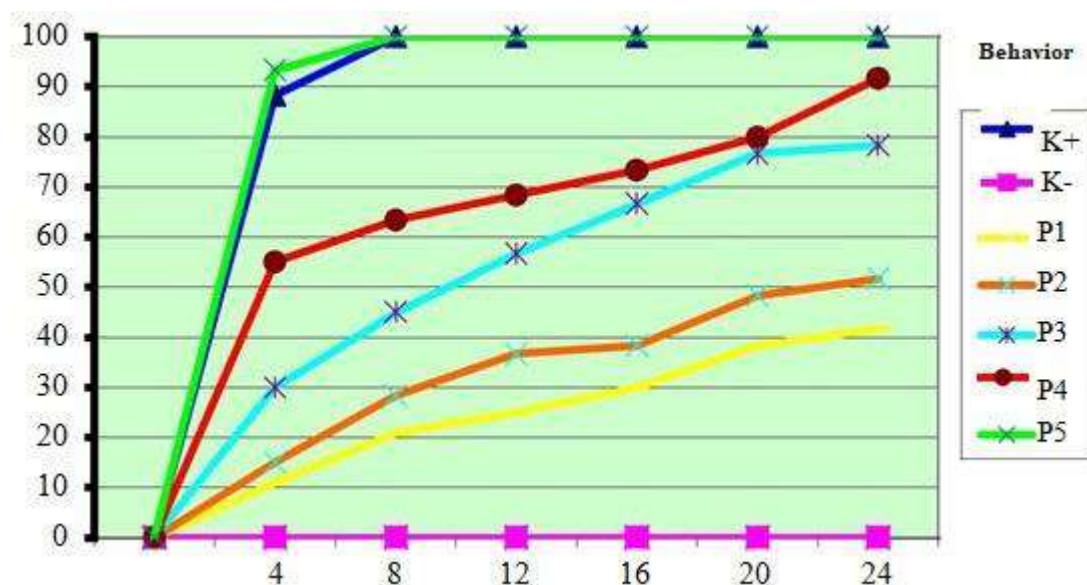


Figure 1. Comparative mortality of Larva *Cx. quinquefasciatus* on observation every 4 hours in a day, K+: Temephos, K-: Aquadest, P1: 250 ppm, P2: 500 ppm, P3: 1000 ppm, P4: 2000 PPM, and P5: 4000 ppm

DISCUSSION

The results of soaking larvae on the negative control (k-) of the larvae mortality rates were 0% for every four hours (Table 1). This means that the larvae were still alive and the addition of aquadest in control treatment did not show any effect on the mortality of larvae. In positive control (k+), the death of Larva instar III *Cx. quinquefasciatus* resulted from the administration of Temephos 1 ppm solution. Temephos is an organophosphate larvicide used to treat water infested with disease-carrying insects. This larvicide has a residual power of approximately one month in the water shelter. As with other organophosphates, temephos affects the central nervous system through inhibition of cholinesterase. In larvae, this results in death before reaching the adult stage. The function of the enzyme cholinesterase is to hydrolyze acetylcholine into choline and sour vinegar (acetic acid) so that if the enzyme is inhibited then hydrolyze acetylcholine does not occur so that the muscles will remain contracted for a long time and will occur continuously, and the insect will eventually die (Nugroho, 2011). So as with other organophosphate compounds, Temephos acts as an anticholinesterase (Nugroho,

2011). Chemical compounds in plants that are potentially biolarvicides, are flavonoids, saponins, tannins, alkaloids, and essential oils (Kristanti et al., 2008). The *C. hystrix* leaf extract has high toxicity of lime leaves (*Citrus aurantifolia*), lime leaves (*C. amblycarapa*), and Balinese citrus leaves (*C. maxima*; Adrianto et al., 2014). The kaffir lime leaves contain essential oils, flavonoids, alkaloids, terpenoids, saponins, and limonoids (Adrianto et al., 2014).

Limonoid compounds are an important natural active component consisting of oxidized triterpenoid components (Khalil et al., 2003). Limonoid causes a bitter taste in orange and has the most potent larvacide effects. The larva cannot be transformed into a pupa because the title of the juvenile hormone in the larvae is not reduced but increases. To be a pupa, larva needed lower amounts of Juvenile hormones. Although the larvae make the skin change to instar IV, the larva will not be able to metamorphosis into the pupa and eventually die (Prijadi et al., 2014). Limonoid can enter into the body of the mosquito larva (*Aedes aegypti*) as a stomach poison. Limonoid goes into digestion through the concentration of the eaten extract concentrations. Insecticides will enter into the digestive organs of insects and are absorbed by the intestinal wall then circulate with blood that will interfere with the metabolism of mosquitoes so that it will lack energy for his life activities that will result in mosquitoes it is seizure and eventually dies (Baskar et al., 2020).

Another substance contained in the leaves of lime that can interfere with insects in digesting food is saponin. Saponin can lower the surface tension of the mucous membrane of the digestive tract larvae. The interaction of the saponin molecules with a layer of larval cuticle will result in damage to the cuticle (Chapagain and Wiesman, 2005). Saponins can also lower the enzyme activity of proteases in the gastrointestinal tract as well as interfere with food absorption (Afidah et al., 2014).

This alkaloid content acts as a stomach poison and contact poison. The alkaloid is a grain of salt so that it can degrade the cell membrane of the digestive tract to enter into and damage cells and can also interfere with the nervous system work of larvae by inhibiting the enzyme work of acetylcholinesterases. Where this enzyme cannot execute its duties in the body mainly forward the delivery of orders to the digestive tract larvae (midgut) so movement cannot be controlled. The occurrence of discoloration in the body of larvae becomes more transparent and the movement of larvae slows down when the touch is stimulated and always bends the body also caused by alkaloid compounds (Ahdiyah and Purwani, 2015).

Tannins are polyphenol compounds that can form complex compounds with proteins. Tannins cannot be digested in the stomach and have a connective power with proteins, carbohydrates, vitamins, and minerals. Tannins can interfere with insects in digesting food because tannins will bind to proteins in the digestive system that required insects for growth so that the digestive process is estimated that become disturbed to the larva *Cx. quinquefasciatus* (Yunita et al., 2009).

The death of Larva instar III *Cx. quinquefasciatus* not only was resulted from the compounds of limonoid but this can also be caused by various ingredients contained in the extract N-hexane lime leaves such as materials that have been mentioned above which can affect the death of larva *Cx. quinquefasciatus*. Therefore, The higher the concentration, the higher the content of the active ingredient contained in the extract of N-hexane lime leaves, which can cause a decrease in digestive activity, causing seizures and disrupt the nervous system and suppress the rate of growth of larvae (Minarni et al., 2013). The content of essential oils that are the secondary metabolite of extracts that are dissolved with aquadest will cause an oil layer to be formed due to differences in polarity. This coating will cover the water surface and siphon. The presence of oil will prevent larvae from taking oxygen, even the coating of oil will stick to the siphon will prevent the diffusion of oxygen towards the tissues, so that oxygen transport is interrupted. The nature of this essential oil can be respiratory poison (Hedayat, 2008).

After soaking the extract of N-hexane citrus leaf, the following kaffir lime signs were observed. In the first 15 minutes, the larvae *Cx. quinquefasciatus* showed a rapidly rising movement, convulsive movements, such as forming the U, weakened body movements, at 2 hours of observation time at concentrations of 4000 ppm and 2000 ppm has begun to show the situation where the larvae are unable to move, stiff, and subsequently die. The results of soaking larvae every 4 hours were observed. Larvacida contact poison can kill enough larva when exposed to external body parts. Then larvae show the sign of ascending movement rapidly (telescopic movement).

The results of the research on the concentration of the extract of N-hexane lime leaves at all treatments except the negative control (k-) were the death of larvae. The mortality rate of larvae at a concentration of 4000 ppm was 93.33%. This concentration is the best concentration of N-hexane extracts of citrus leaves as larvacide. The difference between the numbers of larvae deaths is caused by the concentration of each treatment. This indicates that the higher the concentration and duration of observations of N-hexane citrus leaves of the purut, the higher the active content and the larvasidal power to kill the larva of *quinquefasciatus* mosquitoes.

When compared, concentration 4000 ppm extract N-hexane citrus leaves are equally effective with a solution Temephos 1 ppm in killing the larva instar III *Cx. quinquefasciatus* is the same as indicating the death of 100%. But for the efficiency of the solution temephos is higher in killing the larvae *Cx. quinquefasciatus* then extract N-hexane citrus leaves of kaffir lime with a concentration of 4000 ppm because it only takes 1 ppm (mg/l) of Temephos solution, compared to 4000 ppm (mg/l) extract N-hexane lime leaves to kill 100% larvae for 8 hours in a 24-hour time.

CONCLUSIONS

The findings of this study have confirmed that extracts of N-hexane lime leaves contain active substances that can be larvicidal. Therefore, the results larvacides are considered good when the test material shows the value of mortality between 90-100% of test larvae. In conclusion extract of N-hexane kaffir lime orange leaves have a larvicidal effect on the larva *Cx. quinquefasciatus* in vitro. The optimal concentration of N-hexane extracts of citrus leaves that cause the larval mortality of *Cx. quinquefasciatus* in vitro amounted to 93.33% on 4 hours of observation with a concentration of 4000 ppm.

DECLARATIONS

Competing interests

Authors declare no competing interests.

Authors' contribution

The authors have participated in this study equally.

Ethical consideration

Ethical issues (Including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) have been checked by the authors.

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Effect of Larvicidal Extract N-Hexane Lime Leaves (*Citrus hystrix*) on Larva Instar III Mosquito (*Culex quinquefasciatus*)

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ABSTRACT

Culex quinquefasciatus mosquitoes are a vector of transmission of several types of diseases, such as filariasis, Japanese encephalitis, and dirofilariasis. Larval control is the key strategy of disease control programs caused by vectors around the world because it can prevent larvae to enter the adult stage. Control of mosquito larvae that are often used is chemically controlled. *Citrus hystrix* is a natural plant and contains chemical compounds that have biological activity, such as flavonoids, carotenoids, and limonoids. The present study aimed to determine the effect of citrus leaf extract (*Citrus hystrix*) on the mortality of the larva *Culex quinquefasciatus*. The method used in the current study was a laboratory experimental study method with the experimental design using a completely randomized design. The research plot carried out was the rearing larvae of *Culex quinquefasciatus*, the manufacture of citrus leaf extract, the manufacture of larvicidal material, and the observation phase after treatment. Extract N-hexane *omplet name hystrix* leaf has high toxicity because it contains essential oils, flavonoids, alkaloids, terpenoids, saponins, and Limonoids. The Optimal concentration of N-hexane extracts of citrus leaves that lead to larval mortality of *Culex quinquefasciatus* in vitro amounted to 93.33% on 4 hours of observation with a concentration of 4000 ppm. Extract N-hexane lime leaf has a larvicidal effect on the larva *Culex quinquefasciatus* in vitro.

Keywords: *Citrus hystrix*, *Culex quiquefasciatus*, Extract, Mosquitoes, Larvicide

INTRODUCTION

Indonesia is a tropical country that has optimal humidity and temperature so as to support insect survival. Mosquitoes are the type of insects that coexist with humans but can harm humans because of their role as destructive organisms and disease vectors (vector-borne diseases; Sumantri and Satar, 2015).

Culex (Cx.) quinquefasciatus mosquitoes are the vector of transmission of several types of diseases such as filariasis, Japanese encephalitis, and dirofilariasis (Hadi and Koesharto, 2006). Filariasis is an infectious disease caused by microfilarial worms. This disease can cause disability, psychosocial, and decreased patients' productivity. The number of filariasis cases in Indonesia increases annually. Japanese Encephalitis (JE) is a viral disease that causes encephalitis and is a problem of public health in Asia including in Indonesia. Mosquito control can be done at the larvae stage, but in the adult stage that mosquitoes can fly they can transmit the disease widely (Aradilla, 2009). The control of larvae is the key strategy of vector-borne disease control programs caused by vectors around the world because it can prevent larvae to become adults. The larvae stadium has the longest lifespan of about 10-15 days so that the exposure is also longer, adult stage or adult mosquitoes can also fly so that it can spread the disease widely. The use of insecticide as larvicide can be the most commonly used way for people to control the growth of the vector (Manimegalai and Sukanya, 2014). Control of mosquito larva that has been often used is chemical control, namely by using synthetic chemicals. It can suppress vector populations rapidly. However, control in this way when done repeatedly is less effective because it can cause resistance to larvae, death for animal predatory predators and environmental pollution (Yasmin and Fitri, 2013). Biolarvasida of a plant (vegetable) is considered quite potential replacing synthetic insecticide. Plant insecticides are generally more selective than chemical insecticides because they are directly targeted at the target insect species, but also do not pollute the environment because it is easily degraded by nature and its use is quite safe (Aktar et al., 2009). More than 2400 plant types that belong to 255 families are reported to contain pesticide ingredients. Basil, germanium,

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and zodia belong to a group of mosquito repellent plants due to their distinctive aroma. The plant contains compounds, such as methyl eugenol and some microelements, such as Linalool, Terpinol, Eugenol, Sincol, and Geraniol. In addition, there are other types of mosquito repellent including lavender, zodia, rosemary, fragrant lemongrass, and citrus kaffir lime (Kardinan, 2005). *C. hystrix* is a plant that is found easily by people. Plants derived from this genus of *Citrus* have chemical compounds that have biological activities, such as flavonoids, carotenoids, and Limonoids (Nurhaifah and Sukei, 2015). Limonoid compounds are the teranoriterpen found in the leaves of Citrus lime which is potentially antifeedant to insects, growth regulator, and toxic substances in rice lice, larvacide, antimicrobial, insect repellent, and reproductive inhibitors (Li, 2001). The limonoid compound is an analog of juvenile hormones on insects that serve as a regulator of the growth of larval cuticles (Tunaz and Uygun, 2004). Saponin is also able to become biolarvasida. Saponin compounds in extracts that are eaten by the larvae of *Cx. quinquefasciatus* may irritate the digestive tract of *Cx. Quinquefasciatus*, and damage the cell membrane of the larva. Limonoid, which is an essential oil in orange, can lead to the loss of coordination of the nerve organ of the larva *Cx. Quinquefasciatus* (Pelah et al., 2002). In addition, limonoid has the ability to inhibit the formation of cancer cells, reducing the formation of low-density lipoproteins (LDL) as well as being able to suppress the appetite from insects (Murthy et al., 2021). Therefore, this research is done to find out the benefits of citrus leaves that are thought to have effects on larvae of *Cx. quinquefasciatus*.

MATERIALS AND METHODS

The research was conducted in the entomology laboratory of The Institute of Tropical Disease (ITD), Airlangga University, and the Department of Medicine of Veterinary Faculty of Universitas Airlangga, Surabaya, Indonesia. The material used consists of 420 larvae instar III *Cx. quinquefasciatus* obtained from the laboratory of ITD. Each treatment group used a sample of 20 larvae.

The current study utilized experimental research methods of laboratories with the experimental design using a completely randomized design using simple random sampling. The study was conducted in several phases, namely the rearing phase of the larva of *Cx. quinquefasciatus*, the manufacturing stage of citrus leaf extract of *C. hystrix*, the manufacturing stage of larvacidal material, and the observation phase after treatment. In rearing the larvae of *Cx. quinquefasciatus*, selection was based on the body length (5-6 millimeters, determined by a compound microscope with calibrated eyepiece graticule) and the day after the eggs hatch (four to six days). There was a triangular thickening consisting of many comb-shaped scales, had complete its morphological structure, and clearly, the body could be divided into a part of the head, chest, and abdomen. Prior to the study, the larvae were restored in advance in order to prove that the administration proved optimal. Subsequently, 420 larvae of instar III of *Cx. quinquefasciatus* were divided into seven treatment groups, namely the control group (+) consisting of Temephos 1 ppm + aquadest AD 100 ml solution, the control group (-) consisting of a solution of aquadest 100 ml, treatment 1 entailing 25 mg of citrus leaf extract of kaffir lime + aquadest AD 100 ml, treatment 2 consisting of 50 mg of kaffir lime citrus leaf extract + aquadest AD 100 ml, treatment 3 having 100 mg Citrus leaf extract of kaffir lime + aquadest AD 100 ml, treatment 4 including 200 mg citrus leaf extracts of kaffir lime + aquadest AD 100 ml, treatment 5 consisting of 400 mg of citrus leaf extracts of kaffir lime + aquadest AD 100 ml. The larvacide extract of the kaffir lime citrus leaves was then poured into plastic cups. Then, into the plastic cups that have been contained treatment solution inserted larvae of 20 tails of each glass. Observations were performed every 4 hours for 24 hours to see the dead larvae.

The physical factors measured in the room used during the study included the pH of water, water temperature, and room temperature. Measured water pH ranges from 7.0 to 7.2. The condition is a neutral pH and qualifies for the life of larvae in water. The measured room temperature is 25°C, which is the temperature suitable for larvae growth.

RESULTS

Result of soaking larvae Instar III of *Cx. quinquefasciatus* in various concentrations of treatment for 24 hours obtained the average value and standard deviation of the number of Instar larva III *Cx. quinquefasciatus* every 4 hours observation that can be seen in Table 1.

Based on Table 1 above, it can be noted that there is a significant difference in each treatment of the percentage of mortality of Instar-III Larva ($p \leq 0.05$). However, the results of a comparison of immersion treatment in solution Temephos with extracts N-hexane citrus leaves of 4000 ppm concentrations do not show a noticeable difference. It is different from the immersion comparison of Temephos solution 1 ppm with the N-hexane extracts of citrus leaves with the concentrations of 2000, 1000, 500, and 250 ppm which show noticeable different results. Extract N-hexane citrus leaves of 4000 ppm concentrations at a 4-hour observation have shown an average death of as much as 19 tails so that it can be known that the value (LC_{90}) of extract n-hexane lime leaf is 4000 ppm. More details can be seen in Figure 1.

Based on Figure 1 of the comparative mortality ratio of Larva *Cx. quinquefasciatus* on the observation every 4 hours for 24 hours obtained the result that the relationship between the concentration of N-hexane extracts of citrus

leaves with larvae instar-larva III *Cx. quinquefasciatus* have a positive direction, meaning the higher concentrations of citrus leaf extract kaffir lime, the higher the power of the larva of mosquitoes *Cx. quinquefasciatus*. The increased immersion time at the concentrations of P1, P2, P3, and P4 showed an increase in the number of deaths each time of observation, it showed the time of immersion of N-Hexsan extract material affecting the death process of the mosquito larva *Cx. quinquefasciatus*. In negative control does not encounter the death of larvae it indicates there is no reaction of the use of aqueous solutions.

The results in the experiment indicated a significant difference in each treatment. This means that the death of the larvae of instar III *Cx. quinquefasciatus* was due to the immersion of the larvae in the kaffir lime citrus leaf extract. Insignificant differences in the positive control and the highest concentration of citrus leaf extract kaffir lime resulted from the equation of the effectiveness of citrus leaf extract in the killing of larvae.

Table 1. The results of soaking larvae every four hours

Mortality (%) Error! Bookmark not defined. Groups	Average ± standard deviation					
	4 hours	8 hours	12 hours	16 hours	20 hours	24 hours
K (+)	88.33 ± 2.88	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
K (-)	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
P1	11 ± 5.77	21.66 ± 5.77	25 ± 5	30 ± 5	38.33 ± 7.63	41.66 ± 7.63
P2	15 ± 5	28.33 ± 7.63	36.66 ± 5.77	38.33 ± 7.63	48.33 ± 10.40	51.66 ± 7.63
P3	30 ± 13.22	45 ± 13.22	56.66 ± 14.43	66.66 ± 10.40	76.66 ± 5.77	78.33 ± 7.63
P4	55 ± 5	63.33 ± 7.63	68.33 ± 10.40	73.33 ± 7.63	80 ± 10	91.66 ± 10.4
P5	93.33 ± 2.88	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0

K+: Temephos, K-: Aquadest, P1: 250 ppm, P2: 500 ppm, P3: 1000 ppm, P4: 2000 PPM, and P5: 4000 ppm

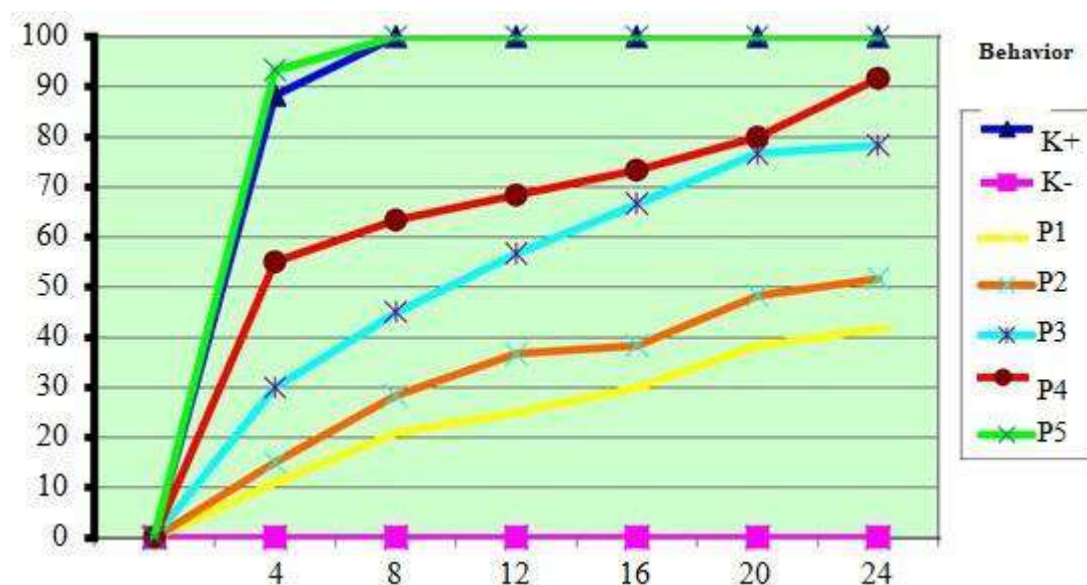


Figure 1. Comparative mortality of Larva *Cx. quinquefasciatus* on observation every 4 hours in a day, K+: Temephos, K-: Aquadest, P1: 250 ppm, P2: 500 ppm, P3: 1000 ppm, P4: 2000 PPM, and P5: 4000 ppm

DISCUSSION

The results of soaking larvae on the negative control (k-) of the larvae mortality rates were 0% for every four hours (Table 1). This means that the larvae were still alive and the addition of aquadest in control treatment did not show any effect on the mortality of larvae. In positive control (k+), the death of Larva instar III *Cx. quinquefasciatus* resulted from the administration of Temephos 1 ppm solution. Temephos is an organophosphate larvicide used to treat water infested with disease-carrying insects. This larvicide has a residual power of approximately one month in the water shelter. As with other organophosphates, temephos affects the central nervous system through inhibition of cholinesterase. In larvae, this results in death before reaching the adult stage. The function of the enzyme cholinesterase is to hydrolyze acetylcholine into choline and sour vinegar (acetic acid) so that if the enzyme is inhibited then hydrolyze acetylcholine does not occur so that the muscles will remain contracted for a long time and will occur continuously, and the insect will eventually die (Nugroho, 2011). So as with other organophosphate compounds, Temephos acts as an anticholinesterase (Nugroho,

2011). Chemical compounds in plants that are potentially biolarvicides, are flavonoids, saponins, tannins, alkaloids, and essential oils (Kristanti et al., 2008). The *C. hystrix* leaf extract has high toxicity of lime leaves (*Citrus aurantifolia*), lime leaves (*c. amblycarapa*), and Balinese citrus leaves (*c. maxima*; Adrianto et al., 2014). The kaffir lime leaves contain essential oils, flavonoids, alkaloids, terpenoids, saponins, and limonoids (Adrianto et al., 2014).

Limonoid compounds are an important natural active component consisting of oxidized triterpenoid components (Khalil et al., 2003). Limonoid causes a bitter taste in orange and has the most potent larvacide effects. The larva cannot be transformed into a pupa because the title of the juvenile hormone in the larvae is not reduced but increases. To be a pupa, larva needed lower amounts of Juvenile hormones. Although the larvae make the skin change to instar IV, the larva will not be able to metamorphosis into the pupa and eventually die (Prijadi et al., 2014). Limonoid can enter into the body of the mosquito larva (*Aedes aegypti*) as a stomach poison. Limonoid goes into digestion through the concentration of the eaten extract concentrations. Insecticides will enter into the digestive organs of insects and are absorbed by the intestinal wall then circulate with blood that will interfere with the metabolism of mosquitoes so that it will lack energy for his life activities that will result in mosquitoes it is seizure and eventually dies (Baskar et al., 2020).

Another substance contained in the leaves of lime that can interfere with insects in digesting food is saponin. Saponin can lower the surface tension of the mucous membrane of the digestive tract larvae. The interaction of the saponin molecules with a layer of larval cuticle will result in damage to the cuticle (Chapagain and Wiesman, 2005). Saponins can also lower the enzyme activity of proteases in the gastrointestinal tract as well as interfere with food absorption (Afidah et al., 2014).

This alkaloid content acts as a stomach poison and contact poison. The alkaloid is a grain of salt so that it can degrade the cell membrane of the digestive tract to enter into and damage cells and can also interfere with the nervous system work of larvae by inhibiting the enzyme work of acetylcholinesterases. Where this enzyme cannot execute its duties in the body mainly forward the delivery of orders to the digestive tract larvae (midgut) so movement cannot be controlled. The occurrence of discoloration in the body of larvae becomes more transparent and the movement of larvae slows down when the touch is stimulated and always bends the body also caused by alkaloid compounds (Ahdiyah and Purwani, 2015).

Tannins are polyphenol compounds that can form complex compounds with proteins. Tannins cannot be digested in the stomach and have a connective power with proteins, carbohydrates, vitamins, and minerals. Tannins can interfere with insects in digesting food because tannins will bind to proteins in the digestive system that required insects for growth so that the digestive process is estimated that become disturbed to the larva *Cx. quinquefasciatus* (Yunita et al., 2009).

The death of Larva instar III *Cx. quinquefasciatus* not only was resulted from the compounds of limonoid but this can also be caused by various ingredients contained in the extract N-hexane lime leaves such as materials that have been mentioned above which can affect the death of larva *Cx. quinquefasciatus*. Therefore, The higher the concentration, the higher the content of the active ingredient contained in the extract of N-hexane lime leaves, which can cause a decrease in digestive activity, causing seizures and disrupt the nervous system and suppress the rate of growth of larvae (Minarni et al., 2013). The content of essential oils that are the secondary metabolite of extracts that are dissolved with aquadest will cause an oil layer to be formed due to differences in polarity. This coating will cover the water surface and siphon. The presence of oil will prevent larvae from taking oxygen, even the coating of oil will stick to the siphon will prevent the diffusion of oxygen towards the tissues, so that oxygen transport is interrupted. The nature of this essential oil can be respiratory poison (Hedayat, 2008).

After soaking the extract of N-hexane citrus leaf, the following kaffir lime signs were observed. In the first 15 minutes, the larvae *Cx. quinquefasciatus* showed a rapidly rising movement, convulsive movements, such as forming the U, weakened body movements, at 2 hours of observation time at concentrations of 4000 ppm and 2000 ppm has begun to show the situation where the larvae are unable to move, stiff, and subsequently die. The results of soaking larvae every 4 hours were observed. Larvacida contact poison can kill enough larva when exposed to external body parts. Then larvae show the sign of ascending movement rapidly (telescopic movement).

The results of the research on the concentration of the extract of N-hexane lime leaves at all treatments except the negative control (k-) were the death of larvae. The mortality rate of larvae at a concentration of 4000 ppm was 93.33%. This concentration is the best concentration of N-hexane extracts of citrus leaves as larvacide. The difference between the numbers of larvae deaths is caused by the concentration of each treatment. This indicates that the higher the concentration and duration of observations of N-hexane citrus leaves of the purut, the higher the active content and the larvasidal power to kill the larva of *quinquefasciatus* mosquitoes.

When compared, concentration 4000 ppm extract N-hexane citrus leaves are equally effective with a solution Temephos 1 ppm in killing the larva instar III *Cx. quinquefasciatus* is the same as indicating the death of 100%. But for the efficiency of the solution temephos is higher in killing the larvae *Cx. quinquefasciatus* then extract N-hexane citrus leaves of kaffir lime with a concentration of 4000 ppm because it only takes 1 ppm (mg/l) of Temephos solution, compared to 4000 ppm (mg/l) extract N-hexane lime leaves to kill 100% larvae for 8 hours in a 24-hour time.

CONCLUSIONS

The findings of this study have confirmed that extracts of N-hexane lime leaves contain active substances that can be larvicidal. Therefore, the results larvacides are considered good when the test material shows the value of mortality between 90-100% of test larvae. In conclusion extract of N-hexane kaffir lime orange leaves have a larvicidal effect on the larva *Cx. quinquefasciatus* in vitro. The optimal concentration of N-hexane extracts of citrus leaves that cause the larval mortality of *Cx. quinquefasciatus* in vitro amounted to 93.33% on 4 hours of observation with a concentration of 4000 ppm.

DECLARATIONS

Competing interests

Authors declare no competing interests.

Authors' contribution

The authors have participated in this study equally.

Ethical consideration

Ethical issues (Including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) have been checked by the authors.

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