



Faculdade de Pindamonhangaba



Jéssica Cordeiro Santos
Leidy Laura de Oliveira Silva

**AVALIAÇÃO DA FITOTOXICIDADE E GENOTOXICIDADE
DE EXTRATOS VEGETAIS COM ATIVIDADE LARVICIDA
UTILIZANDO *Allium cepa* COMO BIOINDICADOR**

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Monografia apresentada como parte dos requisitos para obtenção do diploma de Bacharel pelo curso de farmácia da faculdade de Pindamonhangaba.

Orientador Prof^o Matheus Diniz
Gonçalves Coelho.

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BANCA EXAMINADORA

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A Deus por nos abençoar e capacitar-nos diante das oportunidades e nos conceder perseverança nas dificuldades.

Pelos nossos familiares que nos apoiaram diante das possibilidades e por todo ensinamento na escola da vida.

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“ Dificuldades preparam pessoas comuns
para destinos extraordinários.”

C.S Lewis

Este trabalho foi escrito na forma de artigo científico a ser submetido à revista Panamericana de saúde publica cujas normas estão em ANEXO A.

A parte textual corresponderá ao artigo científico escrito conforme a instrução da revista escolhida.

RESUMO

Os parasitos intestinais são importantes agentes etiológicos para homens e animais, dentre os quais os cães, que consistem em importantes animais de companhia, destacando-se algumas espécies da família Ancylostomidae. A busca por estratégias para descontaminação ambiental, devido a ineficácia de medicamentos alopáticos para o controle de parasitoses como a ancilostomíase, traz o uso de extratos vegetais, mesmo não tendo ainda determinado os impactos consequentes. Assim no presente trabalho objetivou-se avaliar a genotoxicidade e citotoxicidade de 4 extratos vegetais que apresentaram promissoras atividade larvicida e ovicida frente a ovos de *Ancylostoma* sp. à saber: *Allamanda cathartica* L. (Apocynaceae), *Nerium oleander* L. (Apocynaceae) , *Mirabilis jalapa* L. (Nyctaginaceae) e *Brugmansia suaveolens* Willd.(Solanaceae), por meio de ensaios toxicológicos utilizando como organismo teste *Allium cepa*, pelo fato de o mesmo ser de fácil execução e os resultados obtidos serem reprodutíveis e foram avaliados a inibição do crescimento das raízes e efeitos adversos causados aos cromossomos celulares, e extratos foram testados na concentração de 100 ppm. Os extratos vegetais considerados mais promissores foram os obtidos a partir de *M. jalapa* e *B. suaveolens*, já que apresentaram resultados estatisticamente semelhantes ($p > 0,05$) ao grupo controle quanto ao número de células em mitose, porém cabe ressaltar que as células que estavam em divisão, não evoluíram além da fase de prófase, trazendo à tona a necessidade de delineamento de futuros estudos que avaliem concentrações menores, que sejam eficazes quanto à ação ovicida, porém que apresentem resultados mais satisfatórios no que concerne à toxicidade ao meio ambiente.

ABSTRACT

The intestinal parasites are important etiological agents for men and animals, including dogs, which consist of important pets, highlighting some species of Ancylostomidae family. The search strategies for environmental decontamination due the ineffective of allopathic drugs for the control os parasitoses as the hookworm brings the use of plant extracts while not having yet determined associated impacts. As the present work the objective is evaluating the genotoxicity and cytotoxicity of four plants extracts that showed promising larvicidal activity and ovicida front the eggs of Ancylostoma SP. to know: Allamandacathartica l. (Apocynaceae), Neriumoleander l. (Apocynaceae), l. Mirabilisjalapa (Nyctaginaceae) and BrugmansiasuaveolensWilld.(Solanaceae), through toxicological tests using as test organism Alliumcepa, by the fact that the same be easy to perform and the results obtained are reproducible. We evaluated the root growth inhibition and adverse effects caused to cellular chromosomes, and the extracts were tested at a concentration of 100 ppm.The plant extracts considered most promising were those obtained from M. jalapa and B. suaveolens, since presented results statistically similar ($p > 0.05$) to the control group on the number of cells in mitosis, however it is noteworthy that cells that were dividing, not evolved beyond the stage of prophase, bringing forward the need for delineation of future studies that evaluate smaller concentrations that are effective as to action ovicida, however with more satisfactory results with regard to toxicity.

INTRODUÇÃO

As plantas medicinais representam fator de grande importância para a manutenção das condições de saúde das pessoas, contribuindo como uma ferramenta alternativa de escolha, e baixo custo ao tratamento. A fitoterapia representa parte importante da cultura de um povo, sendo também parte de um saber utilizado e difundido pelas populações ao longo de várias gerações. A história do uso de plantas medicinais tem mostrado que elas fazem parte da evolução humana e foram os primeiros recursos terapêuticos utilizados pelos povos.¹

As antigas civilizações têm suas próprias referências históricas acerca das plantas medicinais e, muito antes de aparecer qualquer forma de escrita, o homem já utilizava as plantas algumas como alimento e outras como remédio. Nas suas experiências com ervas, os seres humanos tiveram sucessos e fracassos, sendo que, muitas vezes, estas curavam e em outras matavam ou produziam efeitos colaterais severos. As plantas medicinais são utilizadas mundialmente para o tratamento de doenças, e a maioria delas não foi suficientemente estudada, no que se refere ao seu potencial tóxico.^{1,15}

A atividade biológica de extratos de plantas têm sido largamente estudada, merecendo destaque diversas pesquisas que avaliaram a atividade antiparasitária de extratos vegetais, que tem trazido grandes perspectivas para o controle de parasitoses humanas e veterinárias, dentre as quais a ancilostomíase, que representa uma importante zoonose, particularmente em países em desenvolvimento.¹

Embora ainda não tenha havido avanços significativos na descoberta de novos medicamentos ou produtos para descontaminação ambiental, alguns compostos provenientes do metabolismo dessas plantas, mormente metabólitos secundários, dentre os quais, alcaloides, glicosídeos e taninos, têm demonstrado atividade antiparasitária dose-dependente.²

Neste sentido, destaca-se estudo realizado por Santos et al.², que avaliaram atividade antiparasitária de três espécies de plantas tóxicas, a saber, *Allamanda catártica* L.

(Apocynaceae), *Nerium oleander* L. (Apocynaceae), *Mirabilis jalapa* L., frente a Ancylostomidae. Ao avaliarem extratos hidroalcoólicos desses vegetais, os autores supracitados demonstraram elevado potencial para uso como descontaminantes ambientais, haja vista os mesmos terem apresentado atividade larvicida frente a a *Ancylostoma* sp. obtido de cães naturalmente infectados, na concentração de 50 mg/mL.

Cabe ressaltar que o uso destes extratos para descontaminação ambiental pode acarretar possíveis impactos ambientais decorrentes da exposição de outras formas de vida presentes no ambiente à ação dos mesmos, particularmente por se tratarem de plantas sabidamente tóxicas, o que traz a tona à necessidade do delineamento de pesquisas que determinem o risco ambiental do uso desses extratos no solo.³

Os organismos vivos estão frequentemente expostos a substâncias mutagênicas que podem causar danos celulares. Os danos podem ser induzidos por agentes químicos, físicos ou biológicos que afetam processos vitais como a duplicação e a transcrição gênica, bem como alterações cromossômicas, levando a processos cancerosos e morte celular. Pelo fato de causarem lesões no material genético, essas substâncias são conhecidas como genotóxicas.⁴

Frente a isto, vê-se a necessidade de se avaliar a toxicidade que tais extratos possivelmente poderão ocasionar ao meio ambiente, durante o tratamento empregado, o qual pode ser monitorado pelo uso do sistema teste de *Allium cepa*.⁵

O método de avaliação de alterações cromossômicas em raízes de *Allium* é validado pelo Programa Internacional de Segurança Química (IPCS, OMS) e o Programa Ambiental das Nações Unidas (UNEP) como um eficiente teste par a análise e monitoramento *in situ* da genotoxicidade de substâncias ambientais.^{4,16,17}

O teste *Allium cepa* tem sido utilizado para o estudo dos efeitos de extratos vegetais, visando à detecção de genotoxicidade segundo Fachinetto et al.⁷⁻¹³ Esses sistemas também têm importância no monitoramento da poluição ambiental e avaliação do potencial mutagênico de muitos compostos químicos Ma et al.^{6,15}

Assim sendo, no presente trabalho objetivou-se, por meio do teste *Allium cepa*, efetuar avaliação da citotoxicidade/genotoxicidade de extratos hidroalcoólicos de *Allamanda cathartica* L. (Apocynaceae), *Nerium oleander* L. (Apocynaceae) e *Mirabilis jalapa* L.

MATERIAIS E MÉTODOS

Foram utilizados 4 tipos de espécies vegetais que apresentaram atividade larvicida frente a *Ancylostoma* sp. Objetivou-se avaliar, *in vitro*, a atividade ovicida e larvicida de extratos hidroalcoólicos de dez plantas coletadas na região do Vale do Paraíba ante ovos e larvas de *Ancylostoma* spp. obtidos de cães naturalmente infectados,² cuja identidade botânica foi atestada mediante deposição de exsicatas no herbário SPF da Universidade de São Paulo.

As espécies utilizadas e seus respectivos números de exsicatas foram: *Allamanda cathartica* L. (Apocynaceae) – G. Akisue 041, *Nerium oleander* L. (Apocynaceae) – G. Akisue 040, *Mirabilis jalapa* L. (Nyctaginaceae) – G. Akisue 045, *Brugmansia suaveolens* Willd. (Solanaceae) - G. Akisue 030 & L.M.L. Cardoso.

Para obtenção dos extratos, as partes aéreas de cada vegetal foram processadas na forma de *mix* (folhas, flores e talos), de modo que se obtivesse um extrato para cada espécie. O material vegetal coletado foi processado no laboratório de Farmacognosia (LAFAPLAN) da Faculdade de Pindamonhangaba.

Após secagem em estufa a 45° C e pulverização em moinho de café elétrico (Cuisinart®), foram preparados extratos alcoólicos, utilizando-se álcool etílico (etanol 70° GL) como líquido extrator, pelo método de Soxhlet, segundo as normas da Farmacopeia Brasileira, em sua quinta edição. O extrato alcoólico obtido foi concentrado em aparelho de rotavapor por evaporação do solvente, com obtenção do extrato bruto.

Para realização do teste *Allium cepa*, inicialmente foram selecionados bulbos de *Allium cepa* procurando-se manter um padrão de tamanho, sendo descartados os que

apresentaram algum sinal de deterioração. Foram retiradas as cascas mais externas e os bulbos foram lavados e separados em grupos de cinco unidades, de modo que cada grupo compôs um grupo experimental, a ser exposto ao contato com as suspensões de extratos ou apenas à água de acondicionamento, no caso do grupo controle. Os bulbos permaneceram em contato com os extratos, para germinação das raízes, por 72 horas, tendo sido escolhidos apenas três dos bulbos com maior crescimento destas.

Após este período, com o auxílio de um bisturi e pinça, cortou-se cuidadosamente cada uma das raízes separadamente e estas foram colocadas em recipiente de vidro contendo solução do fixador Etanol/Acético (na proporção de 3:1), em 25° C. Aguardou-se 5 minutos e retirou-se as mesmas, lavando-as em etanol 70% rapidamente. As raízes lavadas foram colocadas em ependorf com solução de etanol 70%, devidamente identificadas e separadas em triplicata, para o armazenamento e conservação, e posterior coloração em lâmina.

As três maiores raízes de cada bulbo foram separadas e submetidas integralmente à reação de Feulgen “en bloc”, sendo a hidrólise ácida realizada em HCl 4 N a 24°C por 75 min. A hidrólise foi interrompida com uma rápida lavagem em HCl 0,1 N, imersas por 40 min no reativo de Schiff, lavadas em água sulfurosa por 3 vezes (5 min cada), lavadas com água destilada conforme metodologia descrita por Barbério et al.⁸

Após o procedimento de coloração, a extremidade mais corada de cada raiz foi utilizada para determinação do índice mitótico, adicionando-se 2 gotas de ácido acético 45% e procedendo o esmagamento entre lâmina e lamínula, seguido de observação em microscópio óptico, no aumento de 400X.

RESULTADOS E DISCUSSÃO

A utilização de biomarcadores como a *Allium cepa* para a avaliação da toxicidade e genotoxicidade tem demonstrado resultados aceitáveis, podendo também ser aplicado para o

estudo de efeitos de citotoxicidade de plantas medicinais, porque as suas raízes ficam em contato direto com a substância testada, permitindo a avaliação de concentrações diferentes.

Segundo Vicentini et al.⁹, a avaliação da presença de alterações no cromossomo e no processo de divisão das células meristemáticas da raiz de cebola é frequentemente utilizada como forma de alertar a população sobre o consumo do produto. Além disso, de acordo com Fiskesjo¹⁰, esse teste tem como vantagem o fato de ser de fácil execução e os resultados obtidos serem rapidamente reproduzíveis.

Matsumoto et al.^{11,14} afirmam que as células da raiz constituem um sistema conveniente tanto para parâmetros macroscópicos (crescimento, deformidade), quanto para parâmetros microscópicos (aberrações cromossômicas). No delineamento experimental do presente trabalho priorizou-se pela avaliação dos critérios microscópicos, onde além de analisar a presença de possíveis aberrações cromossômicas, determinou-se o índice mitótico como forma de avaliar genotoxicidade e citotoxicidade.

Para Leme e Morales^{12,20}, dois conceitos são necessários ao entendimento dos estudos de monitoramento com *Allium cepa*, a) aberrações cromossômicas (AC) são alterações cromossômicas em qualquer estrutura ou no número total de cromossomos, podendo ocorrer tanto espontaneamente, como resultado da exposição física ou de agentes químicos e b) índice mitótico (IM) que é o número total de divisão de células no ciclo celular.

No presente trabalho dois fatores que são indicadores de toxicidade foram observados, à saber: a presença de micronúcleos e a inibição do índice mitótico. No que diz respeito ao primeiro critério, não houve diferença significativa ($p > 0,05$) entre o número de micronúcleos identificados nos grupos que foram expostos aos extratos testados, em relação ao observado no grupo controle, indicando deste modo, que, na concentração de 50 mg/mL, os extratos avaliados não representam risco ambiental.

De acordo com Gravonski^{13,18,19}, o sistema de teste de micronúcleo em raízes da espécie *Allium cepa*, é definido como sendo um dos melhores para estudos de monitoramento

ambiental e mutagenicidade de plantas medicinais, por sua sensibilidade e exatidão, e, porque as raízes da *A. cepa* possuem processo de divisão celular similar aos do homem.

Já no que concerne a inibição do índice mitótico, na fase de prófase nenhum dos extratos vegetais avaliados apresentou um maior potencial tóxico, haja vista o fato de que não se observou diferença significativa no número de células em divisão ($p > 0,05$), quando comparado ao observado no grupo controle.

Por fim, ressalta-se que muito embora todos os extratos não tenham apresentado potencial tóxico quando comparados ao grupo controle, na fase de prófase, observou-se por meio dos resultados obtidos, que todos os mesmos induziram inibição no índice mitótico, já que não foram detectadas células em outras fases do processo de divisão mitótica, exceto no grupo controle conforme APÊNDICE A e B o que estabelece que todos são considerados potencialmente citotóxicos.

Sabe-se que todos os vegetais avaliados no presente trabalho são considerados tóxicos (Santos et al.², porém o fato de extratos obtidos destes na concentração de 50 mg/ mL, já terem se mostrado eficazes e potencialmente úteis para o controle preventivo de parasitoses intestinais, destaca-se a potencialidade do desenvolvimento de pesquisas que visem determinar concentrações que sejam eficazes porém com uma capacidade reduzida de causar impactos ambientais, bem como comparar estes impactos com os decorrentes do uso de produtos industriais.

Por meio da realização do teste de *Allium cepa*, os extratos hidroalcoólicos de *M. Jalapa*, *A. catartica*, *B. suaveolens*, *N. oleander*, são considerados potencialmente citotóxicos pelo fato de terem inibido parcialmente o índice mitótico, porém o fato de estes não terem induzido genotoxicidade, através da contagem de micronúcleos, destaca o potencial uso como descontaminantes ambientais.

REFERÊNCIA BIBLIOGRAFICA

- 1 Tomazzoni MI, Negrelle RRB, Centa ML. Fitoterapia popular: a busca instrumental enquanto prática Terapêutica. Rev Fito popular a busca instrumental.2006;1(15):115-21.
- 2 Santos IA, Souza FJMA, Akisue G, Coelho FAS, Coelho MDG. Avaliação da atividade ovicida e larvicida de dez extratos vegetais frente *Ancylostoma* ssp. Rev Patol Trop, 2013;2(42):209-216.
- 3 Cabrera GL, Rodriguez DMG. Genotoxicity of soil from farmlandirrigated with wastewater using three plant bioassays. *Mutat Res.*1999;211-214
- 4 Bagatini DM, Silva FCA , Tedesco BS Uso do sistema teste de *Allium cepa* como bioindicador de genotoxicidade de infusões de plantas medicinais. Rev. Bras. Farmacogn.Braz J. Pharmacogn; Jul./Set, 2007;17.
- 5 Costa RMA, Menk CFM. Biomonitoramento de mutagênese ambiental. Biotecnologia: ciência e desenvolvimento.2000;(3):24-26.
- 6 Ma TH, Xu Z, Xu C, McConnell H, Rabago EV, Arreola GA, Zhang H. The improved *Allium/Vicia* root tip micronucleus assay for clastogenicity of environmental pollutants. *Mutat Res* 334.1999 ;185-195.
- 7 Fachinetto JM, Tedesco, SB. Atividade antiproliferativa e mutagênica dos extratos aquosos de *Baccharis trimera* (Less.) A. P. de Candolle e *Baccharis articulata* (Lam.) Pers. (Asteraceae) sobre o sistema teste de *Allium cepa*. Rev Bras Pl Med - Botucatu.2009;4(11):360-367.
- 8 Barbério AAB, Barros LA, Voltolini JCB, Mello MLS. Evaluation of the cytotoxic and genotoxic potential of water from the River Paraíba do Sul, in Brazil, with the *Allium cepa* L. test. Braz. J. Biol;2009;69(3):837-842.

- 9 Vicentini VEP, Camparoto ML, Teixeira RO, Mantovani MS. Averrhoa carambola L, Syzygium cumini(L) skeels and cissus sicyoides L: Medicinal Herbal tea effects on vegetal and test systems. Acta Scientiarum. 1999;(23):593-598.
- 10 Fiskejo G. *Allium* test II: Assessment of a chemical's genotoxic potential by Recording aberrations in chromosomes and cell divisions in roots tips of *Allium cepa* L. Environmental Toxicological and Waters Quality. International Journal. 9;1985:235-241.
- 11 Matsumoto, TS; Marin-morales, MA. Mutagenic potential of the water of a river that tannery effluent using the *Allium cepa* test system. Cytologia. 2004;69: 399-408.
- 12 Leme DM, Marin Morales MA, *Allium cepa* test in environmental monitoring: A review on its application. Mutation Research. 2009;682(2):71-81.
- 13 Barbério AA, Voltolini A, Barros JLL. Avaliação preliminar da citotoxicidade e genotoxicidade da água da bacia do rio Tapanhon (SP- Brasil) através do teste *Allium cepa*. Revista Brasileira de Toxicologia. 2007;1-2(20):65-72.
- 14 Meneguetti DUO, Adaptação da técnica de micronúcleo em *allium cepa*, para futuras análises de mutagenicidade dos rios da região do vale do Jamari, Rondônia, Amazônia ocidental. Revista Pesquisa & Criação. 2011;2(10):181-187.
- 15 Arraes AIOM, Longhin RS. Otimização de ensaio de toxicidade utilizando o bioindicador *Allium cepa* como organismo teste. Goiás, Goiânia – Brasil; 2012 junho:15.
- 16 Alvim LB, Kummrow F, Beijo LA, Lima CA de A, Barbosa S. Avaliação da citogenotoxicidade de efluentes têxteis utilizando *Allium cepa* L. Taubaté. 2011;2(6):255-265.
- 17 Canto T, Pich CT, Geremias R. Bioensaios de toxicidade em percolados no aterro sanitário do município de Araranguá (Santa Catarina Brasil). Taubaté; revista biociências. 2013;(19):53-60.
- 18 Souza SC, Lacerda MMP, Irazusta PS. Atividade Mutagênica em *Allium cepa* da Água de Irrigação de hortaliças na região de Itapeçerica da Serra-SP. São Paulo. 7.

19 Müller C, Chagas FF, Peres PLTM, Hess CS, Faccenda O, Daloso MD. Potencial Fitotóxico de Algumas Espécies Gleicheniaceae sobre *Allium cepa* L. Revista Brasileira de Biociências, Porto Alegre.2001 jul;(5), supl.2:45-47.

20 Barbério A, Efeitos citotóxicos e genotóxicos no meristema radicular de *Allium cepa* exposta a água do rio Paraíba do sul.Campinas; 2008;p 98.

ANEXO

ANEXO A - Normas da Revista Panamericana de Saúde Publica.

A. Objectives and Readership

The Pan American Health Organization (PAHO) is an international agency that specializes in public health. It is made up of 35 Member States, three Participating States, one Associate Member, and two Observer States. Its secretariat, the Pan American Sanitary Bureau (PASB), is also the Regional Office for the Americas of the World Health Organization (WHO).

The *Revista Panamericana de Salud Pública/Pan American Journal of Public Health (RPSP/PAJPH)* is a multilingual (English, Spanish, Portuguese) publication that in 1997 replaced the *Boletín de la Oficina Sanitaria Panamericana* and the *Bulletin of the Pan American Health Organization*. Like its predecessors, the *RPSP/PAJPH* offers researchers in the Region of the Americas a scientifically validated, peer-reviewed outlet for public health research findings. It also catalogs the conceptual, social, and political trends indicating the general direction of public health in the countries of the Americas and conveys the decisions and initiatives of PAHO relating to its fundamental purposes: to promote and coordinate the efforts of the countries of the Americas directed toward improving health, fighting disease, prolonging life, and stimulating people's physical, mental, and social development. In addition, the *RPSP/PAJPH* distributes information on the public health activities carried out in PAHO's Member States with the cooperation of the PAHO technical programs.

The *RPSP/PAJPH* is indexed in *Current Contents/Social & Behavioral Sciences*, *Social Sciences Citation Index*, *Index Medicus/MEDLINE/PubMed*, *EMBASE/Excerpta Medica*, *DIALOG*, *LILACS*, *SciELO Salud Pública*, and many other bibliographic databases as *Rev Panam Salud Publica*. Some 7 000 copies per month are distributed to health science professionals, technicians, researchers, professors, and students, both in the Americas and other parts of the world. It is also available in the leading biomedical libraries. The *RPSP/PAJPH* also

has its own interactive website (<http://journal.paho.org/>), where its full contents are available for downloading free of cost. The journal's full contents can also be accessed electronically through SciELO Salud Pública (<http://www.scielosp.org>) at no cost, and through Ingenta (www.ingentaselect.com) at a cost that will depend on the user's country of residence.

B. Contents of the RPSP/PAJPH

The *RPSP/PAJPH* contains materials related to public health in the Region that reflect PAHO's main programmatic areas: health and human development, health promotion and protection, development of health systems and services, environmental health, and prevention and control of diseases. This content is divided into the following sections:

1. Editoriales/Editorials. They deal with the journal itself, specific articles within the journal, or public health issues. Editorials reflect the personal opinions of the individual writing them, who may be an editorial staff member or an independent author. They should always bear the author's signature.

2. Reflexiones del Director/From the Director. Written by the Director of PAHO, this section is published from time to time to communicate the policy and strategic direction of the Organization and the public health priorities in the Region of the Americas.

3. Artículos/Articles. These are original research reports, literature reviews, or special reports on subjects of interest to the Region. Papers presented at meetings and conferences do not necessarily qualify as scientific articles. Studies of clinical cases and anecdotal accounts of specific interventions are not accepted. In general, articles intended for publication as a series on various aspects of a single study are not acceptable either. In general, pieces that have been published previously, in print or electronically (e.g., the Internet), in the same or similar format, will not be accepted. Any instance of such prior publication must be disclosed when the manuscript is submitted, and authors must provide a copy of the published document. On occasion, short communications are published that convey innovative or promising techniques or

methodologies or preliminary results of special interest.

4. Opinión y análisis/Opinion and Analysis. In this section individual authors present their reflections and opinions on topics of interest in the sphere of public health.

5. Temas de actualidad/Current Topics. This section includes descriptions of national and regional health initiatives, projects, and interventions, and of current epidemiological trends, especially relating to diseases and health problems of major importance. Unlike articles, current topics pieces do not reflect original research. However, the same rules concerning prior publication of articles also apply with current topics pieces.

6. Instantáneas (in Spanish only). This section has summaries of the results of studies recently published in prominent English-language journals, as well as press releases from the WHO and other major international public health organizations.

7. Publicaciones/Publications. This section offers brief summaries of current publications dealing with various aspects of public health. Readers are invited to submit reviews of books on subjects within their area of expertise, with the understanding that the reviews will be edited. Each book review should be no more than 1 500 words in length and should describe the book's contents objectively, while approaching the following essential points: the book's contribution to a specific discipline (if possible, as compared to other books of its kind); the quality of the paper, type, illustrations and general format; the kind of narrative style; and whether it makes for easy or difficult reading. The author's professional background and the type of reader the book is addressed to should also be briefly described.

8. Cartas/Letters. Letters to the editor that clarify, discuss, or comment in a constructive manner on ideas expressed in the *RPSP/PAJPH* are welcomed. Letters should be signed by the author and specify his or her professional affiliation and mailing address.

Guidelines for manuscript submission

The Pan American Health Organization holds the copyright to material published in the *RPSP/PAJPH*. Manuscripts are accepted with the understanding that they are original works that have not been published, (in print or electronically, e. g., Internet), or submitted for publication elsewhere, in part or in whole, and that in the future they will not be published or submitted elsewhere without the express authorization of PAHO. Any instance of prior publication in print or electronic format (e.g., the Internet), in the same or similar form, must be disclosed at the time the manuscript is submitted. Authors must provide a copy of the published document.

A. General Criteria for Manuscript Acceptance

The selection of material for publication is based on the following criteria: suitability of the subject for the journal and the importance of the subject matter for the Organization and the Member States; scientific soundness, originality, currency, and timeliness of the information; applicability beyond its place of origin and across the Region; compliance with the standards of medical ethics governing experimentation with human and animal subjects; respect for the Member States and the peoples they represent; a balance of topics and geographic origin of the information; and coherence of the design (a logical statement of the problem and a plan to achieve the objective of the study). Original research should follow the "IMRAD" format (Introduction, Materials and Methods, Results, and Discussion) (see Section II.I). Shortcomings in this regard invalidate all the information and are grounds for rejecting the manuscript. Acceptance or rejection of a manuscript is based on the objective selection process described in Section II.P.

The authors alone are responsible for the views expressed, which may not necessarily reflect the opinion or policy of PAHO or its Member States. The mention of specific companies or of certain manufacturers' products does not imply that they are endorsed or recommended by PAHO in preference to other ones of a similar nature.

B. Specifications

In general, the *RPSP/PAJPH* follows the "Uniform requirements for manuscripts submitted to biomedical journals: Writing and editing for biomedical publication" (revision of November, 2003), developed by the International Committee of Medical Journal Editors. These guidelines are also known as the "Vancouver Style" (see the Bibliography).

The following paragraphs give practical instructions and illustrative examples to prepare a manuscript.

C. Submitting the Manuscript

Manuscripts should be prepared using *Microsoft Word* (or *Excel*, *Power Point*, or other graphics software for the illustrations) and submitted through Manuscript Central™ (ScholarOne, Inc.), which is the online manuscript submission and peer review system employed by the *RPSP/PAJPH*. The site may be accessed via a link provided on the *RPSP/PAJPH*'s webpage (<http://journal.paho.org/>), or directly through Manuscript Central at <http://mc.manuscriptcentral.com/rpsp>

Authors who have difficulty using Manuscript Central should phone our office at 202-974-3046 or, if they speak English, contact Manuscript Central's help line at 1-434-817-2040, extension 167 for calls within the United States and at 011-434-817-2040 for international calls.

Authors will be notified by e-mail that their manuscript has been received. Authors can view the status of their manuscripts at any time by entering Manuscript Central's "Author Center".

D. Language

The *RPSP/PAJPH* publishes articles in English, Spanish, and Portuguese, but manuscripts are accepted in any of the official languages of PAHO (English, French, Portuguese, and Spanish). *Authors should write in their native language, since the inadequate command of a foreign language blurs the meaning of the text and is at odds with scientific precision.*

The *RPSP/PAJPH* reserves the right to publish the text in a language different from the original

and will publish original research articles in only one language.

The titles of references should never be translated. Authors should also refrain from translating the names of institutions unless an official translation exists.

E. Copyright

When an article is submitted through Manuscript Central, the submitting author is required to acknowledge a statement specifying that the text, or a similar one, has not been published before in print or electronically and that it will not be submitted to any other journal before the *RPSP/PAJPH* reaches a decision. Any instance of prior publication in any form must be disclosed at the time the manuscript is submitted. Submitting authors must also acknowledge a statement indicating that if the manuscript is accepted for publication in the *RPSP/PAJPH*, the copyright will be held by PAHO.

Authors are requested to give full information about any grant or subsidy received from a commercial entity, other private group, or WHO, PAHO, or other agency to cover the costs of the work on which the article is based.

Authors are responsible for obtaining permission to reproduce any copyrighted material. The manuscript must be accompanied by the original letter granting such permission. This letter should specify the exact table, figure, or text being cited and how it is being used, together with a complete bibliographic reference to the original source (see Section II.K).

F. Length and Form

The entire manuscript, without including tables, figures, and references, must not exceed 15 to 20 double-spaced pages in *Microsoft Word* using 12-pt. characters in Times New Roman or Arial script. All margins should measure one inch (2,4 cm).

Manuscripts not complying with the specifications outlined above will not be accepted. To be certain they are following the standard format of the *RPSP/PAJPH*, authors should both read all the materials in these Guidelines and also review one or two current issues of the journal before

submitting their manuscripts for consideration. In the case of papers translated in their entirety or containing translations of quoted material, a copy of that text in the original language must be attached.

After peer-review (and possible revision), articles will additionally undergo an editorial process that may include, as needed, condensation of the text and deletion or addition of tables, figures, or annexes. The edited version will be sent to the author for approval and for responses to any additional queries from the editor (see below: II.P and II.Q). The journal may refuse to publish any manuscript whose authors fails to answer editorial queries satisfactorily.

G. Title and Authors

The title should be limited to 10 words, if possible, and should not exceed 15. It should describe the article's contents specifically, clearly, and concisely. Ambiguous words, jargon, and abbreviations should be avoided. A good title makes it easy to grasp what the article is about and helps documentation centers accurately catalog and classify the material.

The online manuscript submission system will register the first and last name, institution, and contact information of every author when a manuscript is submitted. All this information should be omitted from the submitted text entirely in order to safeguard the confidentiality of peer review.

Only those who participated directly in the research or the drafting of the article, and are therefore in a position to assume public responsibility for its contents, may be listed as authors. Inclusion of other persons as authors, out of friendship, acknowledgment, or other nonscientific motivation, is a breach of ethics. For these reasons, an article should have a maximum of eight individual authors. The standards for authorship are extensively explained in the documentation on the Vancouver Style (see Bibliography).

H. Abstract

Every original research article or systematic review must be accompanied by a structured

abstract of around 250 words that is divided into the following sections: (a) Objectives, (b) Methods, (c) Results, and (d) Conclusions. Authors should refrain from translating their Portuguese and Spanish abstracts into English, since this is done in our editorial office. Special reports, opinion papers, and "Current Topics" pieces must be accompanied by an unstructured abstract.

The abstract should not include any information or conclusions that do not appear in the text. It should be written in the third person and should not contain abbreviations, footnotes, references to the main text, or bibliographic citations.

The abstract must enable readers to determine the relevance of the article and decide whether or not they are interested in reading the entire text. In fact, the abstract is the only part of the article, besides the title, that appears in such bibliographic information systems as *Index Medicus*.

Short Communications and Current Topics. These pieces should have an abstract that is a maximum of 150 words.

I. Body of the Article

Articles that report on research or studies are usually organized according to the "IMRAD" format: Introduction, Materials and Methods, Results, and Discussion. Updates or literature reviews and special reports may require other types of headings, depending on their content.

Short Communications. In the case of short communications the usual IMRAD subdivision headings are omitted, but their sequence is followed within the text.

J. Footnotes

These clarifications are numbered consecutively and appear in a smaller type size at the bottom of the page on which they are cited. They are used to give the authors' affiliation (institution and department) and address, as well as some unpublished sources of information (see Section II.K.4). They are also used to make clarifications and give marginal explanations that would

interrupt the natural flow of the text. Their use should be kept to a minimum.

K. Bibliographic References

Citations are essential to identify the original sources of concepts, methods, and techniques referred to in the text and that come from earlier research, studies, and experiences; to support facts and opinions stated by the author; and to provide the reader with the bibliographic information needed to consult the primary sources.

Research and Review Articles. For a scientific article, the *RPSP/PAJPH* requires a minimum of 20 bibliographic references that are both relevant and current. Review articles will generally cite more sources.

Short Communications. These pieces will have a maximum of 15 references.

Citation of References. The *RPSP/PAJPH* uses the "Vancouver Style" for references, according to which all the references should be cited in the text with consecutive numbers, between parentheses, in the following way:

"It has been observed (3, 4) that..."

Or: "Several authors (1-5) have said that..."

The list of references must be numbered consecutively in the order in which the citations appear in the text. The list of references or bibliography should begin on a separate sheet, at the end of the manuscript, and the format must follow the instructions given below.

1. Journal Articles. The following information must be provided: author(s), article title (original, not translated), abbreviated journal title (as it appears in Index Medicus/PubMed), year of publication, volume number (in Arabic numerals), issue number, and beginning and ending page numbers. All this information should be given in the original language of the work cited. The examples below illustrate the "Vancouver Style" of reference construction and punctuation.

a. Individual authors: The surnames and initials of the first six authors should be included;

when there are more than six authors, "et al." should follow. Author information should be written using capital and lower case letters, not all capitals (for example, write Ramos AG, not RAMOS AG).

Kerschner H, Pegues JA M. Productive aging: a quality of life agenda. *J Am Diet Assoc.* 1998;98(12):1445-8.

Silveira T R, da Fonseca JC, Rivera L, Fay OH, Tapia R, Santos JI, et al. Hepatitis B seroprevalence in Latin America. *Rev Panam Salud Publica.* 1999;6(6):378-83.

b. Article published in several parts: Lessa I. Epidemiologia do infarto agudo do miocárdio na cidade do Salvador: II, fatores de risco, complicações e causas de morte. *Arq Bras Cardiol.* 1985;44:255-60.

c. Corporate author: If the corporate author is composed of several elements, they should be given in descending order, from largest to smallest. In the case of unsigned articles in journals published by governmental or international organizations, the organization is regarded as the author.

Pan American Health Organization, Expanded Program on Immunization. Strategies for the certification of the eradication of wild poliovirus transmission in the Americas. *Bull Pan Am Health Organ.* 1993;27(3):287-95.

Organisation Mondiale de la Santé, Groupe de Travail. Déficit en glucose-6-phosphate déshydrogénase. *Bull World Health Organ.* 1990;68(1):13-24.

d. Unsigned article in regular section of a journal: World Health Organization. Tuberculosis control and research strategies for the 1990s: memorandum from a WHO meeting. *Bull World Health Organ.* 1992;70(1):17-22.

e. Special types of articles and other materials: Indicate type or format of the work in square brackets.

Brandling-Bennett AD, Penheiro F. Infectious diseases in Latin America and the Caribbean: are

they really emerging and increasing? [editorial]. *Emerg Infect Dis.* 1996;2(1):59-61.

f. Volume with supplement: Shen HM, Zhang QF. Risk assessment of nickel carcinogenicity and occupational lung cancer. *Environ Health Perspect.* 1994;102(suppl 1):275-82.

g. Issue with supplement: Barreiro C. Situación de los servicios de genética médica en Argentina. *Brazil J Genet.* 1997;20(1 suppl):5-10.

2. Books and Other Monographs. The entry should include the surnames and initials of all the authors (or editors, compilers, etc.), or the full name of an institution, followed by: the title, the edition number, the place of publication, the publisher, and the year of publication. When appropriate, notations may be included indicating the volume and pages consulted, and the series name and publication number.

a. Individual author: Pastor Jimeno JC. *Anestesia en oftalmología.* Barcelona: Ediciones Doyma; 1990.

b. Citing the edition: Day RA. *How to write and publish a scientific paper.* 3rd ed. Phoenix, Arizona: Oryx Press; 1988.

c. Corporate author that is also the publisher: World Health Organization. *The SI for the health professions.* Geneva: WHO; 1977.

d. Chapter in a book: Weinstein L, Swartz MN. Pathogenic properties of invading microorganisms. In: Sodeman WA Jr., Sodeman WA, eds. *Pathologic physiology: mechanisms of disease.* Philadelphia: WB Saunders; 1974. Pp. 457-72.

e. Citing the number of volumes or the specific volume: Pan American Health Organization. *Health conditions in the Americas.* 1990 ed. Washington, D.C.: PAHO; 1990. (Scientific Publication 524; 2 vol).

Pan American Health Organization. *Volume II: Health conditions in the Americas.* 1990 ed. Washington, D.C.: PAHO; 1990. (Scientific Publication 524).

f. Volume with a title: Kessler RM, Freeman MP. Ischemic cerebrovascular disease. In:

Partain CL, Price RR, Patton JA. Magnetic resonance imaging. 2nd ed. Vol. 1: Clinical principles. Philadelphia: Saunders; 1988. Pp. 197-210.

g. Published proceedings of meetings, conferences, symposia, etc: DuPont B. Bone marrow transplantation in severe combined immunodeficiency with an unrelated MLC compatible donor. In: White HJ, Smith R, eds. Proceedings of the third annual meeting of the International Society for Experimental Hematology. Houston: International Society for Experimental Hematology; 1974. Pp. 44-6.

h. Unsigned reports and documents: Information should be given only on written reports that readers can obtain. It is important to indicate the exact name of the organization responsible for the document, the full title, place and year of publication, and document number. If possible, the source of the document should be provided. For example:

World Health Organization. Case management of acute respiratory infections in children in developing countries. Geneva; 1985. (WHO/RSD/85.15)

3. Other Published Materials. Generally speaking, when citing other materials, the standards for a book should be followed, that is, specifying: individual or corporate author, title, generic name for the type of material, the place of publication or issue, and the date of publication. For information in an electronic format, the computer system requirements should also be described.

a. Newspaper articles: Torrey S, Schwartz J. Contraceptive tobacco executives admit health risks before Congress. The Washington Post 1998. January 30:A14 (col. 1).

b. Internet and other electronic media: *Internet sites* - Pritzker TJ. An early fragment from Central Nepal [Internet site]. Ingress Communications. Available from: <http://www.ingress.com/~astanart/pritzker/pritzker.html>. Accessed 8 June 1995.

4. Unpublished Materials and Abstracts. The following should not be included as references:

abstracts of articles, articles submitted for publication but not yet accepted and unpublished works that are not easily available to the public. Articles that are unpublished but have been accepted for publication are an exception to this rule, as are those documents that, while still unpublished, can be easily found. Included in this category are theses, and some discussion papers from international agencies.

Kaplan SJ. Post-hospital home health care: the elderly's access and utilization [PhD dissertation]. St. Louis (MO): Washington University; 1995.

Organización Panamericana de la Salud, Programa Regional Mujer, Salud y Desarrollo. Estrategia global, metas y líneas de acción de la cooperación técnica sobre mujer, salud y desarrollo 1992-1993 [photocopy]. Washington, D.C., February 1991.

If it is absolutely necessary to cite unpublished sources that are hard to obtain, they may be mentioned in the text inside parentheses or in a footnote. The citation in the text is treated in the following manner: It has been observed¹ that...

with the corresponding footnote at the bottom of the page:

¹ Llanos-Cuentas EA, Campos M. Identification and quantification of risk factors associated with New World cutaneous leishmaniasis. [Workshop presentation]. At: International Workshop on Control Strategies for Leishmaniasis, Ottawa, 1-4 June, 1987.

¹Herrick JB [and others]. [Letter to Frank R Morton, Secretary, Chicago Medical Society]. Herrick papers. [1923]. Located at: University of Chicago Special Collections, Chicago, Illinois.

If an article has been accepted for publication and is awaiting publication, the reference should appear as follows:

Wood E, de Licastro SA, Casabé N, Picollo MI, Alzogaray R, Zerba E. Beta-cypermethrin-impregnated fabrics: a new tactic for *Triatoma infestans* control. Rev Panam Salud Publica. Forthcoming 1999.

5. Papers Presented at Conferences, Congresses, Symposia, etc. Unpublished papers that have been presented at conferences should be referenced as footnotes within the text. Only those conference papers that have been published in full (not just as abstracts) in official proceedings should be included in the list of references:

Harley NH. Comparing radon daughter dosimetric and risk models. In: Gammage RB, Kaye SV, eds. *Indoor air and human health: proceedings of the Seventh Life Sciences Symposium*; 1984 Oct 29-31; Knoxville, Tennessee. Chelsea, Michigan: Lewis; 1985. Pp. 69-78.

World Health Organization. *Primary health care: report of the International Conference on Primary Health Care*; 1978 Sept; Alma-Ata, Kazakhstan, former U.S.S.R. Geneva: WHO; 1979.

Unpublished conference papers should be given as footnotes to the main body of the article.

6. Personal Communications. These should be included only if they provide essential information that is not available from a public source. Reference to a personal communication should be given inside parentheses in the body of the text—not in a footnote—in the following way:

Dr. D.A. Little, of the Ecology Center of New York, (personal communication, 2 August 1991) has pointed out that...

Without exception, obtain from the source written verification of the accuracy of the communication.

L. Tables

Tables present information—usually numerical—in an ordered, systematic arrangement of values in rows and columns. The presentation should be easy for the reader to grasp. The data should be self-explanatory and should supplement, not duplicate, the information in the text. Tables with too much statistical information are confusing and hard to understand.

Each table should have a brief but complete title so that the reader can easily determine what the table covers. The place, date, and source of the information should also be indicated clearly. The column heads should be as brief as possible and indicate the unit of measure or the relative base (percentage, rate, index), if any. If information is missing because no observations were made, this should be indicated by ellipsis points (. . .). If the data do not apply, the cell should be marked "NA" (not applicable). If you use either or both of these devices, please indicate their meaning with a footnote to the table. Vertical rules (lines) should not be used in tables. There should only be three horizontal rules: one under the title, a second under the column heads, and a third at the end of the table, above any footnotes. Footnotes to a table should be indicated with superscript lowercase letters, in alphabetical order, in this way ^a, ^b, ^c, etc. The superscript letters in the body of the table should be in sequence from top to bottom and left to right. Prospective authors should consult a current sample issue of the *RPSP/PAJPH* to make certain that their tables follow the journal's standard format.

Short Communications. These pieces should have a maximum of two tables or figures.

M. Figures

Figures (i.e., graphs, diagrams, line drawings, maps, and photographs) should be sent in their original format (such as Excel or Power Point). They should be used to highlight trends and to illustrate comparisons clearly and exactly. Figures should be easy to understand and should add information, not repeat what has been stated in the text. Captions should be as brief as possible but also clear and precise. Figures should not have footnotes. If the figure is taken from another publication, the source must be identified and permission to reproduce it must be obtained in writing from the copyright holder of the original publication. The legend of a graph or map should be included as part of the figure itself if there is sufficient space. If not, it should be included in the figure's title. Maps and diagrams should have a scale in SI units (see Section II.O).

Having too many tables and/or figures is expensive, reduces the desired effect, and takes up much space. Therefore, these materials should be chosen carefully. Information should not be duplicated in tables and figures.

N. Abbreviations

As much as possible, abbreviations should be avoided. The first time an abbreviation or acronym is mentioned in the text, the full term should be given, followed by the abbreviation or acronym in parentheses, as with: Expanded Program on Immunization (EPI).

In general, abbreviations should reflect the expanded form in the same language as that of the manuscript. Exceptions to this rule include abbreviations of agencies known internationally in another language (e.g., CELADE, ILPES, ISO) or such internationally recognized abbreviations as SI (Système international units of measure). (See also Section II.O.)

O. Units of Measure

Authors must use the International System of Units (SI), which is based on the metric system ([see "Bibliography"](#)).

It should be noted that in this system the abbreviations of units are not pluralized (for example, use 5 km, not 5 kms), nor are they followed by a period (write 10 mL, not 10mL.) except at the end of a sentence. Numbers should be grouped in sets of three to the left and to the right of the decimal point, with each set separated by a blank space.

Correct style:

12 500 350 (twelve million five hundred thousand three hundred fifty)

1 900.05 (one thousand nine hundred and five hundredths)

Incorrect style:

12,500,350 / 1.900,05 / 1,900.05

P. Selection Process

The manuscript received undergo a selection process through peer review by experts on the

subject in question. In a first review, the editorial staff of the *RPSP/PAJPH* determine whether or not the manuscript meets the general criteria for manuscripts described earlier ([see Section II.A](#)).

A *second review* considers the scientific merit of the document and the usefulness of its publication; the appraisal is performed by a panel of subject experts who review the manuscript independently. Every manuscript is sent to three reviewers.

In a *third review*, based on the results of the evaluation of general criteria, scientific merit, usefulness of its publication, and the opinion of the peer reviewers, a decision is made to: (a) reject the manuscript, (b) accept in with the condition that the author revise it according to the comments and recommendations of the reviewers, or (c) accept it definitely.

In the case of a conditional acceptance, the revised text undergoes a *fourth review* to make certain that the author has responded to the reviewers' concerns. If the problems have been dealt with and resolved, the article is then accepted; if not, it is rejected.

When a manuscript is accepted conditionally, the author must send back with the revised manuscript a detailed explanation of the changes that have been made to address the peer reviewers' recommendations. When disagreeing with some of those suggestions, the author should give a detailed justification of the reasons.

All decisions are communicated in writing to the author as quickly as possible. The time needed to process a manuscript varies depending on the complexity of the subject and the availability of expert reviewers.

Q. Editing and Publication of the Accepted Article

Manuscripts are accepted with the understanding that the publisher reserves the right to make revisions necessary for consistency, clarity, and conformity with the style of the *RPSP/PAJPH*. Manuscripts accepted for publication will be edited and then sent to the corresponding author to respond to the editor's queries and to approve any corrections. If during

this stage the author does not satisfactorily respond to the editor's queries, the journal reserves the right to not publish the manuscript. Authors will not receive galley proofs of the article. To avoid delay in the publication of the corresponding issue, authors are urged to return the edited manuscript, with their approval, by the date indicated in the accompanying message.

R. Author's Copies

As soon as the article is published, 10 copies of the journal issue in which the article appears will be sent to the corresponding author.

Bibliography

Day RA. How to write & publish a scientific paper. 5th ed. Phoenix, Arizona: Oryx Press; 1998.

Huth EJ. How to write and publish papers in the medical sciences. 2nd ed. Baltimore, Maryland: Williams & Wilkin; 1990.

International Committee of Medical Journal Editors. Uniform requirements for manuscripts submitted to biomedical journals: Writing and editing for biomedical publication. (updated November 2003). Available from: www.icmje.org Accessed 27 January 2004.

Iverson C, et al., eds. American Medical Association manual of style: a guide for authors and editors. 9th ed. Baltimore, Maryland: Williams & Wilkin; 1998.

Riegelman RK, Hirsch RP. Studying a study and testing a test: how to read the health science literature. 3rd ed. Boston: Little, Brown and Company; 1996.

Style Manual Committee, Council of Biology Editors. Scientific style and format: the CBE manual for authors, editors, and publishers. 6th ed. Cambridge, New York: Cambridge, New York: Cambridge University Press; 1994.

World Health Organization. The SI for the health professions: prepared at the request of the thirtieth World Health Assembly. Geneva: WHO; 1977.

1 The information is an updated version of the general information for authors that was

published in Vol. 13, No. 1 (January), 2003. This section may be downloaded and printed from the RPSP/PAJPH's website at <http://journal.paho.org/>

APÊNDICE A

Tabela 1. Avaliação do índice mitótico das raízes de *Allium cepa* sob o teste fitotóxico com extratos de atividade larvicida.

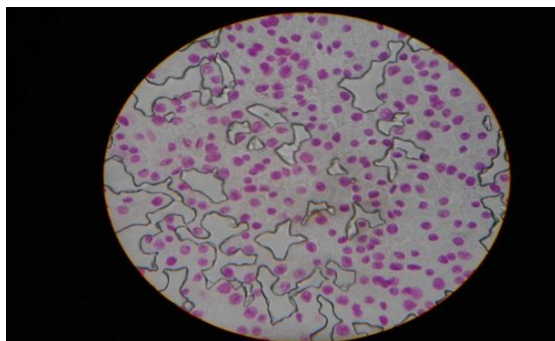
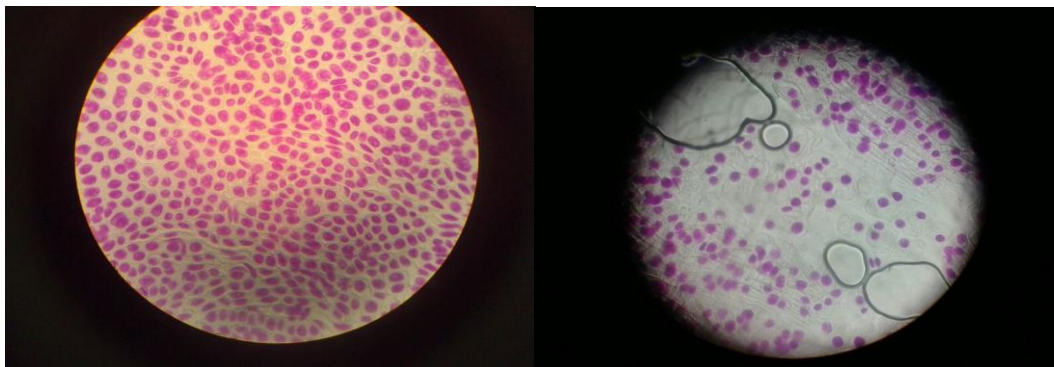
	Controle	<i>M.jalapa</i>	<i>A.catártica</i>	<i>B.suaveolens</i>	<i>N.oleander</i>
Prófase	907	821	445	705	1088
Metáfase	178*	0	0	0	0
Anáfase	42*	0	0	0	0
Telófase	151*	0	0	0	0
Micronúcleos	5	6	0	9	10

* - diferença significativa do grupo controle em relação aos extratos vegetais avaliados

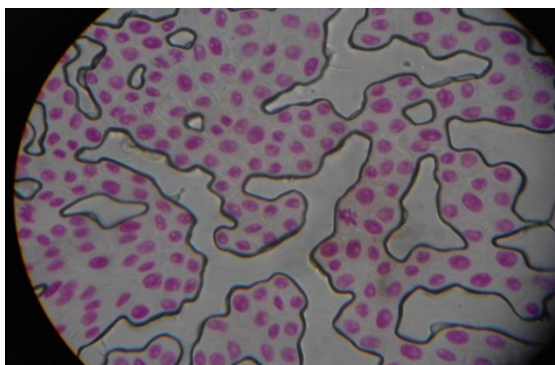
APÊNDICE B

Figuras: Lâmina em microscópio óptico no aumento de 40x :

Inibição do IM no aumento de 40x



Alteração no IM no aumento de 40x



Autorizo cópia total ou parcial desta obra, apenas para fins de estudo e pesquisa, sendo expressamente vedado qualquer tipo de reprodução para fins comerciais sem prévia autorização específica do autor. Autorizo também a divulgação do arquivo no formato PDF no banco de monografias da Biblioteca institucional.

Jéssica Cordeiro Santos e Leidy Laura Oliveira Silva

Faculdade de Pindamonhangaba, Dezembro de 2014.