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ANTIFUNGAL ACTIVITIES OF *PANGIUM EDULE* REINW SEED EXTRACTS INHIBIT THE GROWTH OF *ASPERGILLUS FLAVUS*, PRODUCER OF AFLATOXINS, THROUGH THE *IN-VITRO* TEST

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Pangium edule Reinw seed, *Aspergillus flavus*, Concentration of Extracts, *In- vitro* test, Corn

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ABSTRACT: *Aspergillus flavus* is a famous fungus that produces aflatoxins. Aflatoxins are the most well known toxic and carcinogenic chemical prominent produced in nature. The fungus and aflatoxins are the main concern of the corn consumer. Pangi (*Pangium edule* Reinw) is a potential native plant to reduce infestation of the fungus *A. flavus* to prevent fungal contamination of food or feed. The research was undertaken to assess the antifungal activity of the *P. edule* Reinw extracts of seed against fungus *A. flavus*. Higher concentration is shown as the best concentration to suppress the growth of the *A. flavus* both mycelial growth and emerging new colony. The concentration of the 15% effectively and consistently inhibits *A. flavus* than other treatments. The lower concentration of 0.5%, 0.75%, 1% showed not significantly different over the control. Another higher concentration of the 5% showed that there is no inhibition of the fungus growth. However, the concentration of the 10% indicates also as a good treatment to suppress the fungus growth. Colony appearance after 144 h after inoculation showed that concentration of 15% remains white is different than control that shows mostly green colony. This study exhibits the efficacy of formulated *Pangium edule* Reinw seed in inhibiting *A. flavus* growth.

INTRODUCTION: Mycotoxins contamination on food crops such as aflatoxin in corn is an important food safety problem to both humans and livestock. Aflatoxins are toxic and carcinogenic chemicals compounds produced by fungal species, including *Aspergillus* species. *A. flavus* is one of the important groups of foodborne fungi which are the aflatoxin producers [1, 2, 3].

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A. flavus is a fungus easily to proliferate a wide range of environmental conditions [4]; it can survive temperatures ranging from 12 ºC to 48 ºC, with the optimal growth temperature ranging from 28 ºC to 37 ºC [5, 6] and the fungus can infect crops, particularly maize [6].

Conventional control methods, such as the use of chemical fungicides, are not thoroughly effective in controlling aflatoxins-producing fungi. Moreover, the use of chemical fungicides has disadvantages due to the cost, risk environmental pollution, and disrupt human health [7]. With the efforts for a continued lowering of permissible levels of aflatoxin and pesticides in crops, it is better to apply the natural plant extracts to control aflatoxin-

producing fungi [7]. The benefit of using plants that produce compounds as a source to prevent fungal contamination of the crop is safer and more effective. Moreover, plant extracts effectively proven suppress phytopathogenic fungi and more friendly for the environment [8, 9, 10].

Pangium edule Reinw, which is frequently called Pangi, Keluak, Keluwak, or Keypayang, is one of the commodities of non-timber forest product, and it is a tall tropical tree that grows mainly in Micronesia, Melanesia, and Southeast Asia, including Indonesia [11]. Nearly every section of the plant are toxic including its leaves, barks, and seeds because of the existence of cyanogenic glucosides [12].

Controlling *A. flavus* through bioactive natural product activities is needed; thus the Pangi extracts should be explored more to find out the antifungal activity of the plant. This work aimed to assess the antifungal activity of the *P. edule* Reinw extracts of seed against a broader spectrum fungus *A. flavus*.

FIG. 1: A. *PANGIUM EDULE* REINW, TREE; B. FRUIT; C. SEED

MATERIALS AND METHODS:

Pangi Seed Extraction: The *P. edule* seed was harvested from North Toraja **Fig. 1**, South Sulawesi, Indonesia in 2018. The seed washed on tap water and cut symmetrically.

Moreover, endosperm part was extracted and chopped using blender tool till it becomes smooth. Extraction result of Keluak seed endosperm was put in the container and added with methanol until container near full. Furthermore, the extract was left for five days. After five days, the extracts were filtered using filter paper to separate the small particle of crude extracts, and then the extracts were filtered again to separate crude extracts and solvent using Rotary Evaporator machine.

Medium for *in-vitro* Test: *Pangium edule* seed extracts were mixed in sterilized Potato Dextrose Agar (PDA) medium according to each treatment. The treatments were divided into two different major concentrations:

- Lower concentration:
  a) PDA medium only
  b) The concentration of extract 0.5%
  c) The concentration of extract 0.75%
  d) The concentration of extract 1%
- Higher Concentration:
  a) PDA medium only
  b) The concentration of extract 5%
  c) The concentration of extract 10%
  d) The concentration of extract 15%

Isolation of *Aspergillus flavus*: Isolate of *A. flavus* isolated from infected corn, the corn seed was plated in PDA medium. After 2 days a green colony on medium was placed in a new PDA medium for subculture. To ensure their precise identification, the macroscopic and microscopic characteristics (conidiophores, vesicles, phialides, and conidia) of the isolate was also examined in **Fig. 2**.

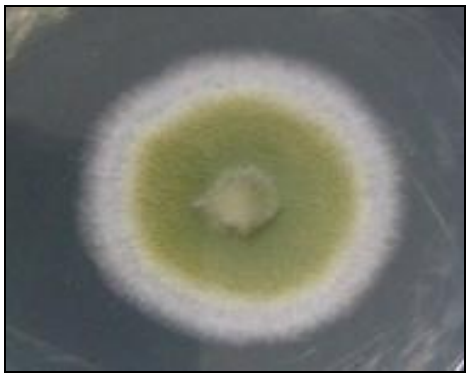

FIG. 2: MACROSCOPIC CHARACTERISTICS OF *A. FLAVUS* ISOLATE

Inhibition Test for *Aspergillus flavus*: Each petri dish was poured 20 ml of the mixed PDA medium and Pangi seed extracts for every concentration and PDA medium only for control. 9-mm of *A. flavus* agar plug was then placed in the center of the petri dish contained both PDA medium + the extracts and PDA medium only for the control. The treated petri dish was kept inside an incubator with the temperature around 30 ºC. The growth of fungus was recorded after 24 h and followed by 24 h the next day. Every treatment replicated four times and for research design using a completely randomized design.

The number of the colony of fungus *A. flavus* was recorded. The data record was started 48 h and resume evaluation every 24 h until the fungus in control petri dish has been spread entirely on the petri dish.

Mycelial growth of fungus *A. flavus* was evaluated as well on 24 h and 48 h post inoculation using measurement tools in millimeter. The evaluation only on 24 h and 48 h after inoculation due to on the preliminary test *A. flavus* colony was easily spread on medium, and the new colony of the fungus emerged around a primary colony. Evaluation of the fungus growth using a formula:

$$D = d1 + d2 / 2$$

D = Growth of fungus
d1 = Vertical growth of the fungus colony
d2 = Horizontal growth of the fungus colony

Statistical Analysis: Influence of *Pangium edule* Reinw extracts inhibits *A. flavus* was determined using analysis of variance (ANOVA). When significant difference detected, means were separated using the Least Significant Difference (LSD) test at 5% probability level.

RESULTS:

Antifungal Activity of Pangi Seed Extracts against *Aspergillus flavus*: The result for antifungal activity of the *P. edule* Reinw was measured by recording the number of the colony emerged and mycelial growth of the fungus *A. flavus*. A preliminary test for lower concentration showed that 0.5%, 0.75%, and 1% not significantly different than control. Meanwhile, the higher concentration of Pangi seed extracts indicated suppression of the fungus *A. flavus* growth **Fig. 3. 4, 5**. Emerging new colony of the fungus on control is numerous than the concentration treatments. On 48 h until 144 h after inoculation, the control consistently shown as the worst treatment, the number of colonies were 20.5, 31.5, 32.0, 32.3 and 32.3 respectively, while the concentration of 5% from 48 h until 144 h, colonies emerged were 17.5, 23.0, 25.3, 26.0, and 26.0. Meanwhile, the number of the colony at 10% started 48 h until 144 h after inoculation is 1.8, 2.5, 6.0, 7.0, and 7.0.

Whereas, the concentration of 15% indicates as the best concentration to suppress and prevent new colony from arising. Started from 40 h until 120 h after inoculation, none of the colony emerged. However, at the last recording on 144 h, 1.3 new colonies emerged. But there is significantly different than other treatments **Fig. 3**. Colony appearance after 144 h after inoculation showed that concentration of 15% remains white, which is only vegetative hypha, is different than control that shows mostly green colony that indicates not only vegetative hypha but also reproductive hypha **Fig. 6**.

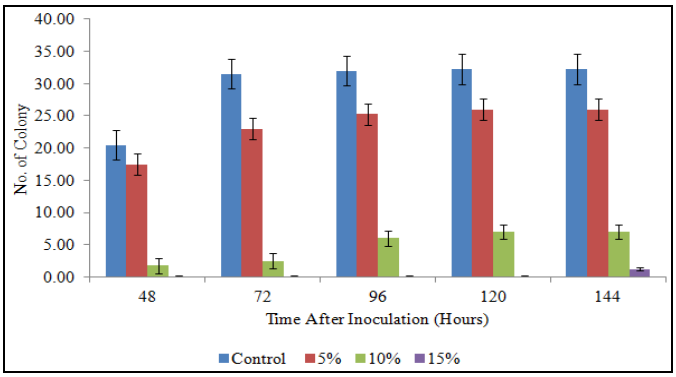

FIG. 3: THE NUMBER OF COLONY OF *A. FLAVUS* ON ANTIFUNGAL ACTIVITY OF THE PANGI SEED EXTRACTS

Mycelial growth on 24 h after inoculation showed that all the concentration treatments are significantly different than control, and among the concentration treatments there is no significant difference. There is no growth of *A. flavus* of the concentration of both 15% and 10% while the concentration of 5% is 0.56 mm, slightly less than control is 6.19 mm. Meanwhile, on 48 h after inoculation indicated that concentration of 15% as the best treatments than other treatments and control followed by 10%, and 5% with growth 10.31 mm, 12.69 mm, 16.38 mm, respectively.

Growth on control faster than the treatments is 18.94 mm. The concentration of extract 15% shown consistently on lowest mycelial growth until 144 h after inoculation **Fig. 4** and **5**.

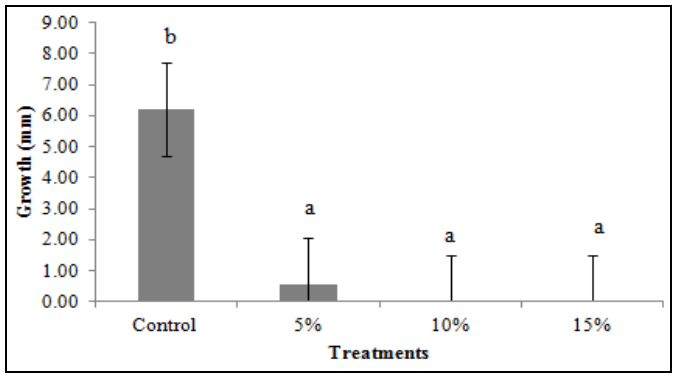

FIG. 4: MYCELIAL GROWTH OF *A. FLAVUS* COLONY 24 h AFTER INOCULATION

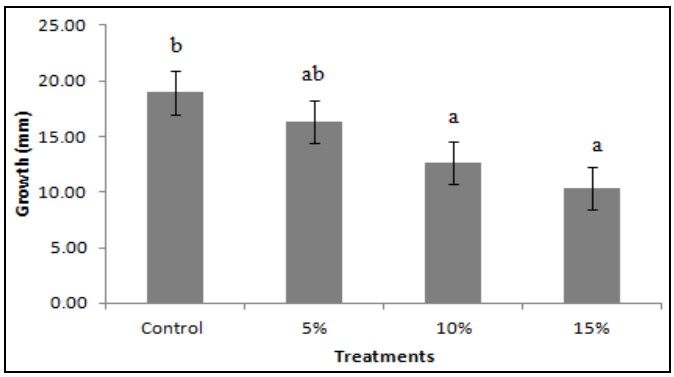

FIG. 5: MYCELIAL GROWTH OF *A. FLAVUS* COLONY 48 h AFTER INOCULATION

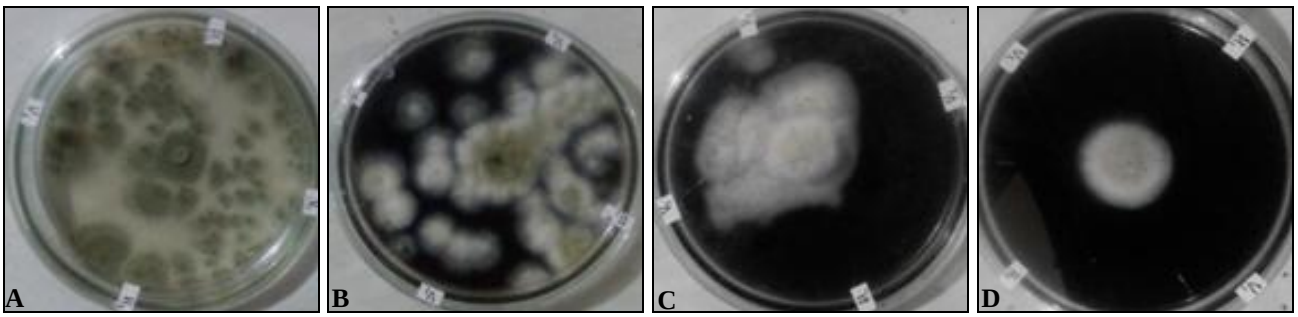

FIG. 6: ANTIFUNGAL ACTIVITY OF PANGI SEED EXTRACTS AGAINST *A. FLAVUS* ON 144 h AFTER INOCULATION (A. CONTROL; B. CONCENTRATION OF EXTRACT 5%; C. CONCENTRATION OF EXTRACT 10%; D. CONCENTRATION OF EXTRACT 15%

DISCUSSION: This study revealed that seed of *P. edule* Reinw extracts had antifungal activities against *A. flavus*. The colony of *A. flavus* has been suppressed both the growth and the spreading. Without application of the Pangi extracts, the fungus able to grow and disperse easily. *A. flavus* is a ubiquitous, saprophytic and cosmopolitan filamentous fungus known can live in different conditions [4, 13] and found abundant on many organic nutrient sources with mono-saccharides and disaccharide [14]. Extracts of *P. edule* Reinw seed could inhibit the growth of *A. flavus* fungus, this result indicates that Pangi seed has antifungal activities. Cyanide compound was found in *P. edule* fruit [15]. Besides, extracts of Pangi seed contains compounds phenolic and alkaloid that effective for antibacterial activities [12, 16]. Moreover, *P. edule* Reinw seed possesses antioxidant activity [12, 11] that could be used as a source of natural preservatives for foodstuffs.

Higher concentration of the extracts showed significant inhibition than lower concentration, 15% of concentration gave the highest antifungal activity followed by 10%. Different concentration of the extracts indicated varied antifungal activity. Higher concentration of the *P. edule* Reinw seed extracts had significant inhibition of the *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus cereus* [16]. The result revealed that secondary metabolites in Pangi seed are capable of inhibiting the growth of the fungus in the higher concentration.

CONCLUSION: This study demonstrates that extracts of the Pangi seed exhibited effective antifungal activities. Therefore, *Pangium edule* Reinw seed extracts could be a promising source of natural preservatives for crops against fungus *A. flavus*.

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CONFLICT OF INTEREST: The authors declare that there is no conflict of interests.

REFERENCES:

1. Kumar P, Mahato DK, Kamle M, Mohanta TK and Kang SG: Aflatoxins: A global concern for food safety, human health and their management. Front. Microbiology 2017; 7: 2170.
2. Faria CB, dos Santos FC, de Castro FF, Sutil AR, Sergio LM, Silva MV, Junior MM and Barbossa-Tessmann IP: Occurrence of toxigenic *Aspergillus flavus* in commercial Bulgur wheat. Food Science and Technology 2017; 37(1): 103-11.
3. Probst C, Bandyopadhyay R and Cotty PJ: Diversity of aflatoxin-producing fungi and their impact on food safety in sub-Saharan Africa. International Journal of Food Microbiology 2014; 174: 113-22.
4. Abbas H, Wilkinson J, Zablotowicz R, Accinelli C, Abel C, Bruns H and Weaver M: Ecology of Aspergillus flavus, regulation of aflatoxin production, and management strategies to reduce aflatoxin contamination of corn. Toxin Reviews 2009; 28: 142-53.
5. Gallo A, Solfrizzo M, Epifani F, Panzarini G and Perrone G: Effect of temperature and water activity on gene expression and aflatoxin biosynthesis in Aspergillus flavus on almond medium. International Journal of Food Microbiology 2016; 217: 162-69.
6. Thathana MG, Murage H, Abia ALK and Pillay M: Morphological characterization and determination of aflatoxin-production potentials of *Aspergillus flavus* isolated from maize and soil in Kenya. Agriculture 2017; 7(80): 1-14.
7. Njoki LM, Okoth SA and Wachira PM: Effects of medicinal plant extracts and photosensitization on aflatoxin producing A*spergillus flavus* (Raper and Fennell). International Journal of Microbiology 2017; 2017: 1-9.
8. Sales MDC, Costa HB, Fernandes PMB, Ventura JA and Meira DD: Antifungal activity of plant extracts with the potential to control plant pathogens in pineapple. Asian Pacific Journal of Tropical Biomedicine 2016; 6(1): 26-31.
9. Shabana YM, Abdalla ME, Shahin AA, El-Sawy MM, Draz IS and Youssif AW: Efficacy of plant extracts in controlling wheat leaf rust disease caused by *Puccinia triticina*. Egyptian Journal of Basic and Applied Sciences 2017; 4: 67-73.
10. Mandiriza G, Kritzinger Q and Aveling TAS: The evaluation of plant extracts, biocontrol agents and hot water as seed treatments to control black rot of rape in South Africa. Crop Protection 2018; 114: 129-36.
11. Suyanto P: The antioxidant activity of the extract of *Pangium edule* reinw. (keluak) seed in cooked ground turkey. The University of Illinois, Thesis in the Graduate College 2012.
12. Chye FY and Sim KY: Antioxidative and Antibacterial Activities of Pangium edule Seed Extracts. International Journal of Pharmacology 2009; 5: 285-97.
13. Hua ST: Biocontrol of *Aspergillus flavus* by Pichia anomala. Microbial Pathogens and Strategies for Combating Them: Science Technology and Education (Mendez-Vilas, A, Ed), Spain 2013; 2: 1067-72.
14. Amaike S and Keller NP: *Aspergillus flavus*. Annual Review of Phytopathology 2011; 49: 107-33.
15. Samudry EG, Sukainah A and Mustarin A: Analysis of the quality of kluwek (*Pangium edule* Reinw) fermented using soil and ash husk. Jurnal Pendidikan Teknologi Pertanian 2017; 3: 25-33.
16. Makagansa C, Mamuaja CF and Mandey LC: The antibacterial activities of pangi kernel extract (*Pangium edule* Reinw) towards *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa* and *Escherichia coli* *in-vitro*. Jurnal Ilmu dan Teknologi Pangan 2015; 3: 16-25.

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