Pathogenicity of entomopathogenic fungi isolates from infected pest crop against dry wood termites *Cryptotermes* sp. (Ioptera: Kalotermitidae)

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**Abstract.** Through exploration at several farming regions in X Koto sub distric, Tanah Datar regency, West Sumatera province (Kandang Sampia, Kayu Tanduak, Panyalaian, Koto Baru), were found 38 fungi isolates. Some isolates (31.58%) pathogenic against drywood termites, *Cryptotermes* sp. Most of those fungi belong to Ascomycota division (*Aspergillus niger*, *Aspergillus* sp. 1, *Aspergillus* sp. 2, *Aspergillus* sp. 3, *Aspergillus* sp. 4, *Penicillium* sp. 1, *Penicillium* sp. 2, *Penicillium* sp. 3, *Penicillium* sp. 4), while 2 fungi did not identified yet (Sp. 1 and Sp. 2). *A. niger* is the most effective fungi to control *Cryptotermes* sp., that indicated by its ability to kill termites (at 10⁷ conidial/ml could kill 93.94% after 7 days infected, higher viability and spore formation), but not differed with *Aspergilus* sp. 1 and *Penicillium* sp. 2. The lowest ability showed by Sp. 1 fungi (at 10⁷ conidial/ml only could kill 60.61% after 7 days infected).

1. Introduction
Dry wood termite is the most feared as silent destroyer wood pest although the amount of damage is relatively slow when compared to the damage cause by ground termites. According to [¹], this is due to termite dry wooden infestation is latent and invisible so it is very dangerous especially if infestation occurs in the main structure of wooden buildings. When earthquake and strong winds the buildings will be prone to collapse and endanger the inhabitant. Various efforts have been made to control drywood termites such as fumigation, heat or cold treatment, spot treatment, dusting and bathing [¹], utilization of organic termiticide [²], inorganic and thermicide, thermicide from microorganisms such as viruses, bacteria [³] and fungi [⁴].

Several types of entomopathogenic fungi have been widely used to control dry wood termite pest. *Metarhizium brunneum* Pech and *Myrothecium roridum* Tode Ex Steudeel were effective to control dry wood termites [⁵], *Metarhizium anisopliae* effective for controlling dry wood termites [⁶], *Beauveria bassiana*, *Aspergillus* sp, *Metarhizium anisopliae*, *Fusarium* sp. and *Myrothesium* sp. can be used to control termites. The application is done by spraying. This way is effective enough to kill termites because if one dead infected termite then other termites will be infected. This happen because termites are insect that have canibal properties, if one termite are dead it will be eaten by other termites, thus the termites will also be infected by fungi [⁷].
Considering the effectiveness of termite control using entomopathogenic fungi and considering the many potentials of entomopathogenic fungi in the field, have not been examined, it has done exploration of various entomopathogenic from various infected plant pest and their effectiveness against dry wood termites.

2. Materials and Methods

Entomopathogenic fungi obtained from various farming regions (Kandang Sampia, Kayu Tanduak, Panyalaian) in X Koto sub distric, Tanah Datar regency, West Sumatera province. Infected pests were put in into film bottles. Spores that come out of infected pests were isolated using inoculation needle and incubated in PDA medium for 5-7 days in room temperature. Pure isolates of fungi then cultured in PDA medium and incubated for 3 weeks. Conidial suspension of fungi got out from PDA medium through shake down the petridish using sterile aquadest that contain 0.05% Tween 80. Those suspensions after that poured into test tube. Serial dilution method used for making suspension to the needs of (10^7 conidia/ml). Haemocytometer is used to count conidial density of fungi.

Pathogenicity test of fungi use drywood termites that collected from infested building in Padang. Pathogenicity testing of fungi isolates conducted before identifying the isolates. Fungi application (10^7 conidial/ml) were done through topical method to 1 soldier and 10 worker termites. Infected termites transferred in to plastic bowl that covered with tissue paper which used as food of termites. Infested termites kept in dark room. Mortality of termites counted everyday for a week. Isolate be said to be pathogenic if can cause mortality > 60% [8]. Isolates that showed pathogenic to drywood termites, then identified by virtue of fungi macroscopic and microscopic characteristics correspond to characteristic that stated by Barneet and Hunter [9].

Physiology characteristic of fungi observation carried out on selected entomopathogenic fungi (could kill termites >80%), including viability and spore formation. To determine fungi viability of selected fungi, as much as 0.1 ml of 10^5 fungi conidia/ml put in PDA medium and then incubated on 24°C. Observation on conidial germinate one day after incubation. Comparison output between germinate conidial amount and conidial total that cast out in medium constitute as fungal viability. Spore formation observation carried out by incubated 0.1 ml of 10^5 fungi conidia/ml in PDA medium on 24°C during 15 days. After that, in petridish 50 ml sterile aquadest poured and shook around five minutes, filtered and diluted three times. Conidial concentration counted by haemocytometer and the average of conidial as compared to another isolates. Fungal colony growth (in diameter) observed from each Petri dish during 15 days with 3 days time gap.

3. Results and discussion

The result of fungi exploration from several farming regions in X Koto sub distric, Tanah Datar regency, West Sumatera province (Kandang Sampia, Kayu Tanduak, Panyalaian, Koto Baru), found 38 fungi isolates (Table 1).

| Table 1. Fungi isolates found on infected insect in Tanah Datar various farming region |
|-----------------------------------------------|
| No. Region | Isolates resource | Amount of isolate | Total |
|---|---|---|---|
|Kandang Sampia| A1| 2 | 11 |
| | A2| 4 |
| | A3| 2 |
|Kayu Tanduak| A4| 3 |
| | B1| 3 | 9 |
| | B2| 2 |
| | B3| 2 |
|Panyalaian| B4| 2 |
Table 1 indicated that there are many fungi that could infect insects in the field in each region. This suggests that the spread of entomopathogenic fungi is very broad. There were 17 isolates of entomopathogenic fungi incorporated three genera namely Metarhizium, Beauveria, and Aspergillus in various types of vegetables rhizosphere in Tanah Datar and Agam Districts [10].

Table 1 also showed that in villages Kandang Sampia and Panyalaian are found more insects infected with entomopathogenic fungi than in other regions. This shows that in both areas the spread of entomopathogenic fungi is wider than other villages. This is thought to be related to the moisture of each region. The epizootic entomopathogenic fungi is strongly influence by climate especially the warm and humid climate [11].

The result of identification of fungi found in infected crops insects are dominated by the fungi included in the Ascomycota division (Aspergillus and Penicillium) (Table 2). Wide variety of fungal pathogens that reported as potential pathogens to termites [12]. Fungal species of at least 11 genera from four fungi families, i.e. Deuteromycetes, Zygomyceyes, Ascomycetes and Mitosporic, success tested against termites [13]. Three genera fungi in Tanah Datar and Agam districts that potential as entomopathogenic fungi (Beauveria, Metarhizium and Aspergillus) [10].

Table 2. Fungi isolates that found in infected insects crops in various region of Tanah Datar and its pathogenicity against drywood termites

| Kind of fungi      | Mortality (%) | Total | Average | Notation |
|--------------------|---------------|-------|---------|----------|
|                    | P1            | P2    | P3      |          |
| Control            | 22.72         | 22.72 | 18.18   | 21.21    | a        |
| sp.1               | 59.09         | 59.09 | 63.64   | 60.61    | b        |
| sp.2               | 68.18         | 63.64 | 68.18   | 66.67    | b        |
| Penicillium sp. 3  | 59.09         | 68.18 | 72.73   | 66.67    | b        |
| Aspergillus sp. 3  | 72.73         | 68.18 | 86.36   | 74.09/    | bc       |
| Penicillium sp. 1  | 72.73         | 81.82 | 72.73   | 75.76    | bc       |
| Aspergillus sp. 4  | 72.73         | 81.82 | 77.27   | 77.27    | c        |
| Penicillium sp. 4  | 72.73         | 90.91 | 68.18   | 77.27    | c        |
Aspergilus sp. 2 81.81 72.73 77.27 231.81 77.27 c
Aspergilus Sp. 5 63.63 90.91 81.82 231.82 78.79 c
Penicillium Sp. 2 86.36 72.73 81.82 240.91 80.30 cd
Aspergilus Sp. 1 90.91 90.91 81.82 263.64 87.88 cd
Aspergilus niger 95.45 90.91 95.45 281.81 93.94 d

Note: Numbers that followed by the same lowercase indicate that there is no significant different on 5% significant level.

Table 2 also showed that form pathogenicity testing all of isolates pathogenic to drywood termites. It means that all isolate that found in infected insect crops from Tanah Datar pathogenic to drywood termites and Tanah Datar fungi potential to be drywood termiticide. According to [12], fungi are the most promising entomopathogens for the development of a microbiological termiticide.

Entomopathogenic fungi seem have differed pathogenicity against drywood termites. Among all of pathogenic isolates, A. niger showed highest pathogenicity to drywood termites even though not so differed with Aspergilus sp 1 and Penicillium sp. The difference of fungi pathogenicity indicated that each of fungi has specific potency in controlling drywood termites. According to [12], biological control agents had tried with varying results. Fungal control agents are promising group of insect pathogens suitable for use as biopesticides for the control of insects. However, limited availability, cost and reliability have hampered the development of such fungal control agents. Host range and specificity has been a problem as well as an advantage; a fungal pathogen that is virulent and pathogenic to one insect species may be ineffective against other species, even those of the same genus. However, some success had demonstrated. Among the Deuteromycetes tested against termites, Verticillium indicum (Petch) Gams and V. lecanii (Zimmermann) Viegas were more virulent to Cryptotermes brevis Walke (Isoptera: Rhinotermitidae) and Odontotermes brunneus Hagen (Isoptera: Termitidae) than Metarhizium anisopliae (Metsch) Sorok (Hyphomycetes) [15].

The ability of A. niger cause highest termites mortality to be related to it’s viability (Table 3), and spore formation ability (Table 4). It means that A. niger could be an alternative biotermicides. Pathogenicity of entomopathogenic fungi determined by several factors including host defense and fungi physiology such as viability, growth rate, spore formation ability and ability to produce enzyme and toxin, and environment [16].

| Kind of Fungi     | Viability | Total | Average | Notation |
|-------------------|-----------|-------|---------|----------|
| Penicillium sp. 2 | 38 40 41  | 119   | 39.67   | a        |
| Aspergillus sp. 3 | 44 47 38  | 129   | 43      | a        |
| Aspergillus niger | 51 59 54  | 154   | 5133    | b        |

Note: Numbers that followed by the same lowercase indicate that there is no significant different on 5% significant level.

Aspergillus niger isolates that found cause mortality to termites known as entomopathogenic fungi with various substances that kill drywood termites. Aspergillus spp., produce aflatoxins B1, B2, G1, and G2 [17].
Table 4. Spore formation ability of selected fungi (conidial density 10^6 conidia/ml)

| Kind of Fungi | Viability | Total | Average | Notation |
|---------------|-----------|-------|---------|----------|
| Penicillium sp. 2 | 11    | 12    | 9       | 32       | 10.67    | a |
| Aspergillus sp. 3 | 13    | 14    | 16      | 43       | 14.33    | a |
| Aspergillus niger | 16    | 15    | 19      | 50       | 16.67    | b |

Note: Numbers that followed by the same lowercase indicate that there is no significant different on 5% significant level.

4. Conclusion

Fungi exploration from several infected insects crops in farming regions in X Koto sub distric, Tanah Datar regency, West Sumatera province (Kandang Sampia, Kayu Tanduak, Panyalaian, Koto Baru), found 38 fungi isolates Fungi isolates that found dominated by Ascomycota division, that is Aspergillus and Penicillum. Aspergillus niger showed most pathogenic to termites than others, that determine by it’s ability to cause termites mortality, viability and spore formation ability.

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