



Effectivity of Inhibition of Oil Palm Empty Fruit Bunches Liquid Smoke Against *Ganoderma boninense* Fungus In Vitro

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Abstract

Oil palm empty fruit bunches (OPEFB) can be processed into liquid smoke through pyrolysis. This study aimed to obtain the best concentration of unpurified liquid smoke from OPEFB in inhibiting the growth of the fungus of *Ganoderma boninense*. OPEFB samples were obtained from PT. Citra Putra Kebun Asri and *G. boninense* were collected from Muara Teweh Plantation. The study was conducted in 2 stages; Analysis of liquid smoke from pyrolysis using the GC-MS method at the Organic Chemistry Laboratory, Faculty of Mathematics and Natural Sciences, Gadjah Mada University; and testing the inhibition of liquid smoke on *G. boninense* in vitro at the Basic Plantation Cultivation Laboratory, Hasnur Polytechnic, and the Microbiology Laboratory of Plantation Products Processing Technology, Muara Teweh Polytechnic. This study was conducted in a completely randomized design with four treatments and six replications. The treatment was the difference in concentration of liquid smoke (1 ml/AC1, 2 ml/AC2, 3 ml/AC3, and control/AC0) in the planting medium, potato dextrose agar (PDA). The results showed that the average yield of unpurified liquid smoke from OPEFB was 34.5%, containing 49.28% acetic acid, 11.57% methyl alcohol, and 9.10% phenol. Application ≥ 2 ml liquid smoke in PDA completely inhibited *G. boninense*'s growth in vitro, starting three days after application.

Keywords: Acetic acid, basal stem rot disease, control, concentration, OPEFB, phenol

Introduction

Oil palm (*Elaeis guineensis*) can produce crude palm oil for cooking oil, industrial oil, and fuel (Setyamidjaja, 2016). Oil palm fruit can be processed into several semi-processed materials, such as crude palm oil (CPO) and palm kernel oil (PKO) (Syahputri et al., 2022).

Indonesia is one of the world's main palm oil producers, apart from Malaysia, Thailand, and Papua New Guinea (Setyamidjaja, 2016). Oil palm plantation areas are located in 26 provinces; Sumatra and Kalimantan, West Java Province, Banten, Central Sulawesi, South Sulawesi, Southeast Sulawesi, West Sulawesi,

Gorontalo, Maluku, North Maluku, Papua, and West Papua. In 2021, Riau Province became Indonesia's largest palm oil producer, with an area of 2.86 million ha or 19.55% of the total area of oil palm plantations in Indonesia (Directorate of Food Crops, Horticulture, and Plantation Statistics, 2021).

When palm oil is extracted and processed, it also generates waste with organic matter, suspended matter, and oils and fats. A large amount of residue and waste is in the form of empty fruit bunches, palm shells, plant stems, fibers, leaves, and others (Shridhar and Adeoluwa, 2009). The Indonesian Plantation Fund Management Agency (2018) states that the most solid waste from oil palm is empty fruit bunches (EFB). At least one ton of processed fresh fruit bunches will produce 583 kg of liquid waste. Furthermore, it can produce as much as 144 kg of solid waste and 64 kg of fiber and shells, and 210 kg of Empty Palm Oil Bunches (EPOB).

Oil Palm Empty Fruit Brunches (OPEFB) waste can be utilized to become useful products. Empty fruit bunches can be processed into valuable products such as liquid smoke (Widiastuti et al., 2022) through pyrolysis (Mohamed et al., 2014). The pyrolysis process of 6 kg of OPEFB produces 3.6 liters of liquid smoke (Kresnawaty et al., 2017b). Liquid smoke produced by pyrolysis of OPEFB contains phenolic compounds, acetic acid, and tar and has a pH of around 3.20. The concentration of 5% phenolic content and 0.454% acetic acid produced from the OPEFB pyrolysis process can be used as coagulants in rubber plants (Evahelda et al., 2021).

Liquid smoke from OPEFB and coconut shells can be used as a pesticide (Silaban et al., 2022). Research conducted by Gani et al. (2014) showed that OPEFB liquid smoke could be used as a fungicide for the fungus *Colletotrichum capsici*, which causes anthracnose in chili peppers. Likewise, in the

study of Wardoyo et al. (2020), liquid smoke from OPEFB has antifungal activity against *Colletotrichum* sp. (WA2). Liquid smoke from OPEFB can also be applied as insecticide for brown planthopper (Soedijo et al., 2015). Based on those data, liquid smoke made from OPEFB is expected to suppress the growth of the fungus *Ganoderma boninense*, the primary pathogen of oil palm plantations. *G. boninense* degrades the lignin component in oil palm trees, which causes stem rot disease (Bharudin et al., 2022). *G. boninense* can attack oil palms at the production and nursery stages. Typical symptoms prior to the formation of the fungus' fruiting bodies are marked by rotting at the base of the stem, causing dry rot in the deep tissue (Semangun, 2008). Susanto et al. (2013) reported that all samples taken for stem rot symptoms in Labuhan Batu Regency, Riau, were *G. boninense*.

Based on Agustina (2020), liquid smoke from coconut shells with a concentration of 20% effectively inhibits the growth of *G. boninense*. *G. boninense* was also controlled using liquid smoke made from empty palm fruit bunches (Mahmud et al., 2021) at a concentration of 5%. The study aimed to produce liquid smoke made from OPEFB, to determine its physical and chemical characteristics, and to determine the effectiveness on the growth of *G. boninense*.

Methods

This research was carried out in 2021 at PT. Hasnur Citra Terpadu, Puting River, South Kalimantan, Basic Laboratory of Plantation at Hasnur Polytechnic and Laboratory of Biochemical Processing Technology of Plantation Products at Muara Teweh Polytechnic, Indonesia.

Method

This research is an experiment consisting of 2 stages. The first stage was making

liquid smoke, and the second stage was testing the inhibition of liquid smoke on the growth of *G. boninense* in vitro. The study was conducted in a completely randomized design (CRD) which focused on differences in 4 concentrations of liquid smoke in potato dextrose agar (PDA) media, namely: 1 ml (AC1), 2 ml (AC2), 3 ml (AC3), and control (AC0), repeated 6 times respectively.

Making liquid smoke

Production of OPEFB liquid smoke through pyrolysis (Ni'mah et al., 2019). Empty palm fruit bunches are obtained from the palm oil mill of PT. Hasnur Citra Terpadu (Tenera Variety), then dried and chopped to reduce size. After drying, 650 grams of OPEFB were put into a modified pyrolysis tube from a 12 kg gas cylinder at a temperature of 2000C for 1-1.5 hours. The pyrolysis process stops when no liquid smoke comes out of the discharge hose (Figure 1). This process was repeated three times.



Figure 1. Production of liquid smoke: a. Oil palm dried bunches (EFB), b. EFB in pyrolysator

Providing *G. boninense* Isolate

The first step was to sterilize the surface of the symptomatic plant parts using 70% alcohol. Then cut with a size of about 1 cm x 1 cm, then wash with sterile water. The cut sections were transferred into 0.5% NaOCl solution for 1 minute, then 96% alcohol for 15-30 seconds. The piece is then cut again with a smaller size (0.5 x 0.5 cm). Then inoculated into potato dextrose agar (PDA) medium and incubated at room temperature (28°C) for five days. After five days of incubation and already

having mycelium growing around the pieces of the fruiting bodies, then the hyphae that are farthest from the pieces are taken and inoculated on a new PDA medium to be purified (Purnamasari, 2012).

Control Treatment

The range of concentrations was tested using the poison food technique to test the antifungal ability of each treatment against *G. boninense*. The PDA media was placed into the petri dish without adding liquid smoke. Then, *G. boninense* isolate was inoculated by placing in the middle of the petri dish. The growth of the fungus was observed for 3, 6, and 9 days, and the diameter was measured using a digital caliper. This method refers to Ganapathy et al. (2021).

Treatment using liquid smoke

Liquid smoke was put into 100 ml of PDA medium, which was still liquid, with 1 ml, 2 ml, and 3 ml of each treatment. The liquid smoke and PDA media mixture were put into a petri dish. Furthermore, pathogenic fungi were inoculated in each of these treatments by placing a piece of the colony in the middle of the petri dish. The next step is to observe the inhibition of each treatment for three days, six days, and nine days. Each observation was carried out by measuring the growth diameter in PDA media. The liquid smoke was first filtered with Whatman cat no 1001 125 filter paper and can immediately be used as research material. This method refers to Ganapathy et al. (2021).

Parameter Observed

Characteristics of EFB Liquid Smoke

The liquid smoke obtained was subjected to a color test and a pH test. Determination of color using the visual observation method with the sense of sight directly on the test sample and determining the pH using a pH meter (BSN, 2021). Based on SNI liquid smoke, the color of liquid smoke

for quality 2 is yellow to brown, and the pH ranges from 2.76 -4.50.

The Content of Phenol, Acetic Acid and Methyl Alcohol

The liquid smoke characteristic test was analyzed at the Organic Chemistry Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Gadjah Mada. The characteristics analyzed were yield, phenol content, and acetic acid content. The method used is using Gas Chromatography-Mass Spectrometry (GC-MS). Determination of the content of phenol, acetic acid and methyl alcohol to show the potential of liquid smoke as an antimicrobial. Calculation of the percentage content of the three organic compounds is based on the chromatogram table from the results of sample testing, then read the description of the peak report from the chromatogram.

Growth of *G. boninense*

The growth of *G. boninense* was measured by calculating the diameter of the colony of *G. boninense* isolates using a digital caliper on a petri dish (This method refers to Dendang, 2015). Measurements were taken on the 3rd, 6th and 9th day. The measurement results show growth with increasing the value of the colony diameter and vice versa

Inhibition rate of liquid smoke on the growth of *G. boninense*

Inhibition was observed 3, 6, and 9 days after *G. boninense* inoculation. The inhibitory rate was calculated by measuring the growth diameter of *G. boninense* from each treatment. The data obtained is used to calculate the percentage inhibition of diameter growth (PIDG) of liquid smoke on the growth of *G. boninense*, with the following formula (Fernanda, 2021):

$$\text{PIDG (\%)} = (K-P)/K \times 100\% \dots\dots\dots (1)$$

K = growth diameter of *G. boninense* in control, P = growth diameter of *G. boninense* in liquid smoke treatment.

Data analysis

The measured data were analyzed using analysis of variance and further analysis using the least significant difference (LSD) test at the α level of 5%.

Results

Characteristics of EFB Liquid Smoke

The results showed that the liquid smoke produced from OPEFB was dark brown, with a pH of 3.5. The pyrolysis liquid smoke used in this study was unpurified liquid smoke (Figure 2).

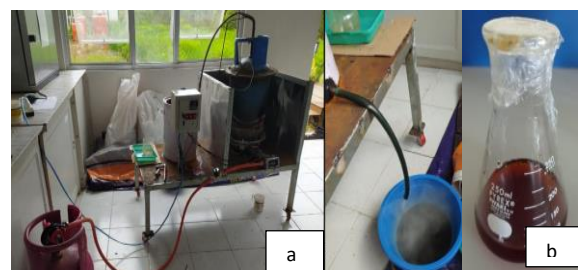


Figure 2. Pyrolysis: a. The process of making liquid smoke, b. Pyrolysis liquid smoke

The Content of Phenol, Acetic Acid and Methyl Alcohol

The analysis using the GC-MS method showed that phenol and acetic acid compounds are among the compounds abundant in OPEFB liquid smoke, namely 49.28% acetic acid and 9.10% phenol. The methyl alcohol (11.57%) was also detected as a compound in this liquid smoke (Figure 3).

Growth of *G. boninense*

In this study it can be seen that the observations on the 3rd, 6th and 9th day, liquid smoke treatment can affect the growth of *G. boninense*. This can be seen from the growth rate of *G. boninense* (colony diameter) which differed to the control (without liquid smoke) and the liquid smoke treatment.

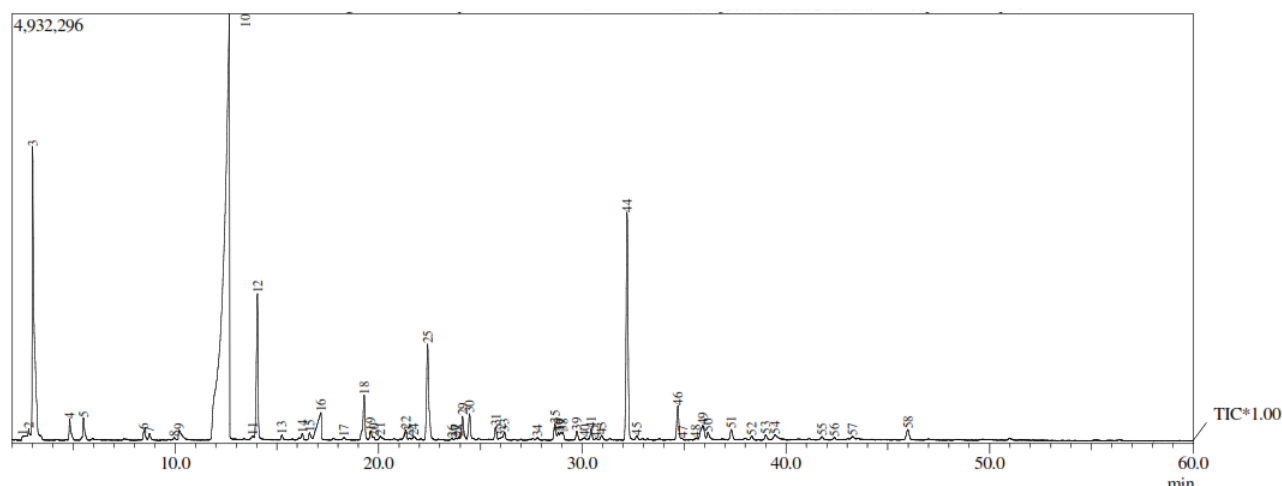


Figure 3. GC-MS results for the content of acetic acid, phenol, and methyl alcohol

At the time of observation, the PDA with 1 ml liquid smoke treatment showed slower growth of *G. boninense* than the control, while

2 ml liquid smoke and 3 ml liquid smoke had no growth at all (Table 2).

Table 2. Growth diameter of *G. boninense* in the treatment of liquid smoke concentrations on the 3rd, 6th, 9th day

Concentration	Growth diameter <i>G. boninense</i> (mm)/observation (day)		
	3	6	9
Control (AC0)	52.8 ± 11.30a	82.97 ± 3.89a	96.83 ± 0.05a
1 ml (AC1)	33.43 ± 0.15b	57.7 ± 12.73b	86.57 ± 8.54b
2 ml (AC2)	0 ± 0.00c	0 ± 0.00c	0 ± 0.00c
3 ml (AC3)	0 ± 0.00c	0 ± 0.00c	0 ± 0.00c

Numbers in the same column followed by different letters show significant differences based on the LSD (least significant different) test at the α test level of 5%

Inhibition Rate of Liquid Smoke on the Growth of *G. boninense*

Liquid smoke from pyrolysis made from OPEFB has shown inhibition on the growth of *G. boninense*. On the 3rd, 6th and

9th day of observation, based on the results of the analysis of variance, it was shown that the liquid smoke treatment had a significant effect on inhibiting the growth of *G. boninense* (Table 3).

Table 3. Inhibition of *G. boninense* in liquid smoke concentration treatment on day 3, 6, 9 after application

Concentration	Inhibition rate (%)/observation (day)		
	3	6	9
Control (AC0)	0 ± 0.00a	0 ± 0.00a	0 ± 0.00a
1 ml (AC1)	36.68 ± 1.87b	30.45 ± 15.34b	10.67 ± 8.76b
2 ml (AC2)	100 ± 0.00c	100 ± 0.00c	100 ± 0.00c
3 ml (AC3)	100 ± 0.00c	100 ± 0.00c	100 ± 0.00c

Numbers in the same column followed by different letters show significant differences based on the LSD (least significant different) test at the α test level of 5%

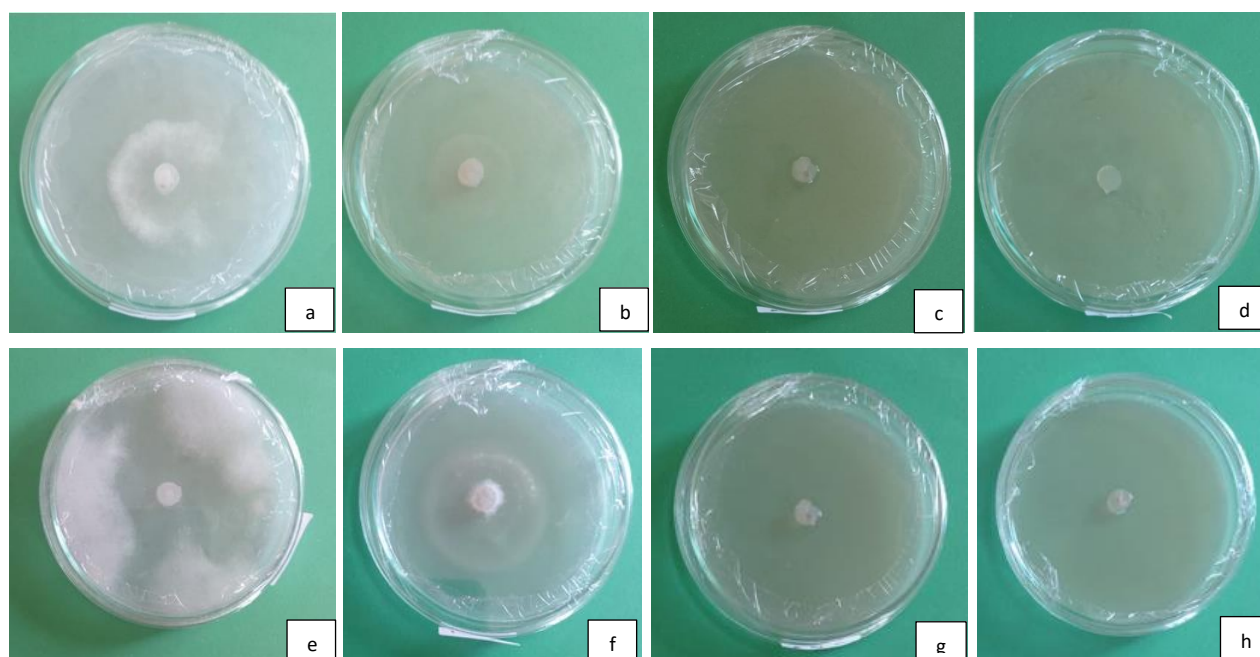


Figure 4. Inhibition of liquid smoke on the growth of *G. boninense* on the 3rd day of observation: a. control (AC0), b. AC1 treatment (1 ml), c. AC treatment 2 (2 ml), d. AC treatment 3 (3 ml), and on the 9th day: e. control (AC0), f. AC1 treatment (1 ml), g. AC treatment 2 (2 ml), h. AC treatment 3 (3 ml)

Discussion

The results showed that the liquid smoke OPEFB used was quite good (Figure 2). The color of liquid smoke after being filtered is dark brown. The color is the same as the liquid smoke processed by Widiastuty et al. (2022). However, in contrast to the color of liquid smoke from OPEFB made by Wardoyo et al. (2020) and Maulina et al (2017), which are deep red in color. The pH of liquid smoke in this study was 3.5. This is the same as the research by Kresnawaty et al (2017b). In addition, research by Sari (2018) and Karima (2014) shows the pH of liquid smoke from OPEFB is 3.20, while the results of research by Fauziati et al. (2021) and Pratama et al (2022) show the pH of liquid smoke from OPEFB is 3.8 and 4.

The results of the study showed the pH of pyrolysis liquid smoke was 3.5. This is indicated by the presence of acetic acid in liquid smoke 49,28%. The presence of acetic

acid in the liquid smoke added to the PDA media probably increased the pH of the growth medium for *G. boninense*. According to Syafina et al. (2020), acetic acid can destroy the outer membrane of the cell wall of organisms, inhibit the synthesis of macromolecules, increase the production of antimicrobial peptides in host cells, and consume microbial energy. It is known that the growth and morphology of fungi are affected by the pH of the growth medium. Organic acids can lower pH values and affect growth by acidifying cells which in turn will consume a lot of energy to maintain intracellular pH homeostasis.

The results showed that liquid smoke from OPEFB contains organic compounds with the highest content acetic acid (49.28%), methyl alcohol (11.57%), and 9.10% phenol (Figure 3). The results of this study are inversely to Ni'mah et al. (2019) where the highest content was 56.856% phenol and

10.793% acetic acid. However, the content of organic compounds in the EFB from the research results was higher than that of Haji et al. (2013) where the chemical content of liquid smoke from OPEFB for acetic acid (16%) and phenol (3.56%), and Oramahi et al. (2010) 6.31 % acetic acid, 3.63 % phenol.

The liquid smoke component made from OPEFB contains antimicrobial ingredients. According to Ni'mah et al. (2019), components that play an important role as antimicrobials in liquid smoke are phenol and acetic acid. Based on the research results on the chemical content test of liquid smoke from OPEFB, it obtained 49.28% acetic acid content and 9.10% phenol content. Phenolic compounds are secondary metabolites of plants which generally exhibit a wide range of physiological properties, such as involved in defense against ultraviolet radiation or aggression by pathogens (Jee et al., 2015). Phenol contained in liquid smoke plays a major role as an anti-fungal (Winarni et al., 2021). The results of the research on the 3rd day of observation in the PDA + 1 ml liquid smoke treatment showed that the growth of *G. boninense* was inhibited compared to the control (figure 4). There is growth but the mycelium is thin compared to growth in the control treatment. Surendran et al. (2017), conducted research using the "poison food" test method, by testing the growth of *G. boninense* on agar medium in media containing phenolic compounds. The results showed that the mycelium of *G. boninense* which grew on media containing phenolic compounds showed damage. The results of microscopic observations showed that the hyphae of *G. boninense* were less in density, distorted, had reduced branches and experienced lysis. In addition, phenolic compounds have inhibited the growth and production of wood degrading enzymes. Likewise with research by Kresnawaty et al.

(2017a) that the addition of phenolic acid as an inhibitor reduces the activity of lignolytic enzymes from *Ganoderma*.

Liquid smoke made from OPEFB at PT. Citra Putra Kebun Asri has the potential to control the growth of the oil palm pathogenic fungus of *G. boninense*. Based on the results of measuring the growth diameter of *G. boninense* (Table 2) and testing the inhibition of liquid smoke (Table 3), it can be seen that the best concentration of liquid smoke was in the PDA with 2 ml liquid smoke. Observation of inhibition on day 3, 6 and 9 shows a consistent value of 100% inhibiting the growth of *G. boninense*. The results of this study are in line with research conducted by Agustina (2020), where the inhibition of *G. boninense* is 100% in the treatment of liquid smoke made from coconut shells. Likewise, the research by Sharip et al. (2016) that using liquid smoke (10%) from oil palm mesocarp fiber can inhibit 100% the growth of the mycelium of *G. boninense* UPM13 fungus which causes oil palm basal stem rot disease. However, there are differences with the results of research by Mahmud et al. (2021), Liquid smoke made from OPEFB grade 2 has an inhibitory effect on the growth of *G. boninense* of 94.33% in the treatment of liquid smoke with a concentration of 5%. This proves in research, PDA treatment with 2 ml of liquid smoke grade 3 can suppress the growth of *G. boninense* 100%.

The inhibition of *G. boninense* can also be influenced by the carbonyl contained in the liquid smoke. Some of the ingredients contained include carbonyl for example 2(5H)-furanone, and 2Furancarboxaldehyde (CAS) Furfural, as well as others. Sharip et al. (2016) stated that various furan compounds in the fractions might cause interference with various cell enzymatic functions and consequently, cell death. This is due to the presence of aldehyde moieties such as

furfural. In addition, the content of liquid smoke such as alcohol can also inhibit microbial growth. According to Mc Donnell (2017) showed that alcohol through its reactive hydroxyl group can cause the deposition and denaturation of proteins and macromolecules of microbial cells through the formation of hydrogen bonds.

Conclusion

This study concluded that the average yield of unpurified liquid smoke from oil palm empty fruit bunches (OPEFB) was 34.5%, that containing 49.28% acetic acid, 11.57% methyl alcohol, and 9.10% phenol. The treatment of ≥ 2 ml liquid smoke in PDA completely inhibited *Ganoderma boninense*'s growth in vitro, starting from 3 days after application.

Declaration

Author contribution

Rahmawati is the main contributor and corresponding author for this paper. Cica Riyani and Zuliyan Agus NM Majid are the co-authors. All authors read and approved the final paper.

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Competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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