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Retrospective Analysis of Equine Traumatic Injury: Patterns and Insights

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ABSTRACT

Traumatic injury is a catastrophic and second most frequent emergency condition that is significantly labor intensive and expensive to treat, raising a welfare concern in the equine industry. Even though the incidence is high, there is still a lack of data addressing the prevalence of these traumatic injuries, particularly in Malaysia. Therefore, this study aims to describe the occurrence and patterns of the traumatic injuries experienced by the subpopulation of horses in Malaysia. The records of horses reported to the University Veterinary Hospital, Universiti Putra Malaysia between January and December 2023 were reviewed to determine traumatic injury cases and all data were analysed descriptively. Among 422 patient medical records, 99 horses (23.0%) were reported to have sustained a total of 107 traumatic injuries. Majority of the injuries were reported to occur in the stable (26.2%), while being ridden (18.7%), and during the competition (18.7%), in which most of the cases were associated with wounds (60.7%), but the occurrence of fractures (9.3%) and injured ligaments or tendons (15.0%) were also high. Occurrence of being kicked by another horse (11.2%) and penetration by sharp objects (11.2%) were the most common mechanisms of injury, whereby forelimbs (38.8%) and hindlimbs (29.8%) were injured the most, commonly at the distal aspect. This data will enable horse owners and veterinarians to employ better traumatic injury preventative strategies.

Keywords: Horse, prevalence, risk factor, traumatic injury

INTRODUCTION

Traumatic injury refers to the damage or harm inflicted upon the structure or function of the body as a result of an external or internal force, encompassing both physical and chemical components (Owen et al., 2011). Traumatic incidents in horses can occur in general-purpose and sport horses. These injuries encompass a wide range of severity, from minor cuts and scrapes caused by saddle material, falls, kicks, or bites to more serious traumas that involve fractures of bone or torn ligament and tendon. In cases of catastrophic musculoskeletal injury, there is a high probability horses will be resorted to humane euthanasia to alleviate the suffering and painful condition. Most recent research has focused particularly on injury in sport or performance horses, with special attention given to identifying the causes and risk factors that predispose to injuries in polo ponies (Inness & Morgan, 2014), dressage horses (Murray et al., 2010), jumping horses (Gibson et al., 2023), and racehorses (Crawford et al., 2021; Rouette et al., 2021). Briefly, tendon injuries were perceived as the major problem in polo ponies in which the hard

provides an important risk factor for this incidence, followed by wounds and splint bone fracture. In racehorses, traumatic musculoskeletal injuries were the most common cause of death reported in Canada and the United Kingdom, comprising over 70.0% of racehorse fatalities (Allen et al., 2017; Physick-Sheard et al., 2018; Rosanowski et al., 2016). The high occurrence of traumatic injuries in sport horses has significant implications for training losses and wastage in horses across various disciplines (Mekete, 2022; Reilly & Bryk-Lucy, 2021). Nonetheless, general – purpose horses are also at risk of experiencing the incidence of traumatic injuries. A study in the United Kingdom has shown that 40.0% of general–purpose horses also sustain a traumatic injury (Owen et al., 2011). Sixty–two percent (62%) of the incidence occurred in the field, while 13.0% was during ridden exercise. The breed of horse, ownership duration, turn out environment, and stabling all time during spring were identified to be the factors associated with the increased risk of traumatic injury. Despite injuries that take place during competition or being ridden, trauma in horses is also attributed to the incidence of bite and kick, which prevalence was found to be 8.0% and 21.6% in Swiss equine populations (Knubben et al., 2008). Apart from chasing, rearing, and mounting, biting and kicking are also typical horse behaviours and are associated with aggressive, threatening, submissive, and avoidance behaviour to preserve a long–lasting relationship, while some might happen by mistake as a result of exuberant excitement behaviour. Therefore, ensuring a stable group hierarchy and providing sufficient space in pasture and paddock have been identified as crucial elements in preventing these injuries (Knubben et al., 2008). Besides, trauma to horses in transport vehicles during a long journey also occurred frequently. The majority of research has focused on horses intended for human consumption that are transported by road using commercial companies, in which the incidence of injuries varies from 1.6% to 33.0% (Roy et al., 2015a, 2015b). In Australia, 45.0% of survey participants reported an incidence of traumatic injury related to both commercial and non-commercial horse transportation (Padalino et al., 2016). Additionally, a retrospective survey of injury during non-commercial transportation has reported that equine behaviour was the attributed cause of 56.0% of incidents reported and most incidents occurred during the first hour of travel (Hall et al., 2020). Wounds, bone fractures, and injuries to ligaments as well as tendons were commonly associated with trauma in horses. Wounds at the limbs were the most frequently seen in the form of abrasions, lacerations, or incisions. A survey in New Zealand demonstrated a high occurrence of skin wounds, particularly on the distal limb, which were costly and timeconsuming to manage and may lead to decreased performance, retirement, or euthanasia (Theoret et al., 2015). On the other hand, bone fractures can occur in any part of the body. Kick injuries have been reported to cause fractures of the second and fourth metacarpal and metatarsal bones, followed by bones of the skull, proximal and distal phalanx, pelvis, and tibia (Donati et al., 2018). The fracture can be either simple fractures, comminuted fractures, fissures, or depression fractures, as well as the least seen, were Salter–Harris fractures. Despite the high occurrence of traumatic injury to the equine population worldwide, the data on

. traumatic injuries are still limited, particularly in Malaysia. Therefore, the aims of the study are to highlight the occurrence of equine traumatic injuries by determining the frequency and assessing the patterns associated with the incidence. These findings will provide valuable insights for the horse owners and direct the veterinary teams to offer more specific care and enable prevention strategies for the horses.

MATERIALS AND METHODS

The case records of all horses reported to the University Veterinary Hospital, Universiti Putra Malaysia were reviewed. Data from January to December 2023 were selected and analysed for the occurrence of the equine traumatic injury. Cases that were confirmed or suspected traumatic injury incident had taken place were selected. A confirmed traumatic injury was determined based on the keyword “Traumatic injury” indicated in the record and as well as based on the case history. Meanwhile, records for all horses with a suspected traumatic injury were examined and deemed eligible for inclusion in this study, in which any of the following criteria were met: the presence of one or several lacerations, incision, ulceration, or abrasion wounds, swelling of the affected region, pain upon palpation, lame upon walk or trot and fracture of a bone. Cases were excluded if there was insufficient or no clear evidence of a traumatic injury based on the case report. Case information was collected detailing the date of occurrence, signalment of the horse (breed, sex, age, uses), aetiology of injury, and physical examination findings. Additional information regarding the cases was obtained through verbal communication with the veterinarian responsible for the cases. Data were entered and managed in Microsoft Excel 2021 ((Microsoft Corporation) and IBM SPSS Statistics version 27 software (IBM Corporation) was utilized to analyse the data and determine the frequency distribution. The analysed data were reported as frequencies and percentages. The distribution of traumatic injury was divided into groups in accordance with the risk factors. Outcomes of each aspect were evaluated and were expressed as a percentage of the total number of outcomes from all aspects by using tables and charts.

RESULTS

A comprehensive analysis of 422 patient medical records yielded a total of 107 traumatic injury cases reported from 99 horses (23.0%) from January until December 2023 as illustrated in Figure 1. Among these, 8 horses sustained multiple injuries, whereas the remaining 91 horses were reported to have suffered only a single injury each. The average number of cases that occurred was 9 cases in a month, which ranged from a minimum of 6 cases seen in February, March, October, and December to the highest of 15 cases reported in June. Table 1 shows the number and percentage of traumatic injury according to sex, age, breed and horse's uses classifications. Of all horses examined, there were comparable number of mare and geldings recorded with small portion of stallion and filly. Among them, there were a higher number of adults reported with injuries compared to foal and geriatric horse. The breed distribution comprised of Thoroughbred, Polo Ponies, Warmblood, Arabian, Criollo, Friesian and local pony. Most

horses were used for sport and companionship. However, in 15 horses, there was no information regarding their uses at the time of injury reported.

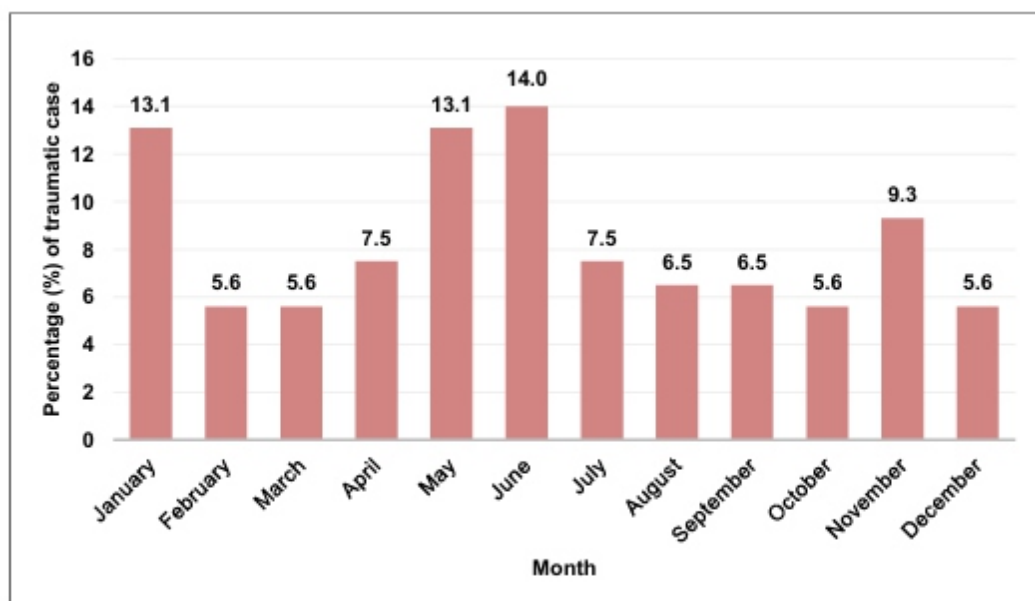


Figure 1. Monthly distribution of traumatic injury cases in horses, from a total of 107 cases, presented to the University Veterinary Hospital, Universiti Putra Malaysia, from January to December 2023

The locations of traumatic injury incident took place are shown in Table 2. The occurrence was mostly reported to occur at the stable or stall. This was followed by injuries that occurred during competition, as well as during ridden for training and leisure rides, which had similar occurrences in this study. Traumatic injury also takes place when the horse is released into the paddock. Rarely, traumatic injury was also reported to occur during transportation. However, in two cases, the location of the injury was not documented in the medical Table 3 displays the causes of traumatic injury sustained by the horses over the year. The most frequent causes of injury were a kick from another horse and also penetration by sharp objects. Other causes involved falling or stumbling, crashes and hits that during polo matches, and also improper saddle or bit used. Some horses also exhibited self-inflicted injury. This self-mutilation encompasses behaviours such as biting, stomping, kicking, rubbing, and lunging into objects. Wounded during transportation occurred rarely. However, about half of the cases, the cause of injury could not be determined. Table 4 presents the various types of traumatic injuries sustained by horses that were presented to the hospital throughout the year 2023. Of all the trauma cases, injury presented with single or multiple cutaneous wounds contributes to the highest cases reported to the hospital. This wound includes laceration, abrasion, or incision wounds that involve any parts of the body. Injury toward the ligament and tendon accounted for 15.0% of the cases, particularly involving the ligament and tendon of the limbs. This was followed by fractures which mostly occur to the splint bone, where the lateral aspect of the limbs was injured in approximately 86.0% and the medial aspect in 14.0% of the cases. Rarely there were also fractures toward the sesamoid bone, nasal bridge, and tooth. Trauma to the eye attributed to the 8.4% of the cases reported. The horses were also suffering from puncture wounds which resulted

Table 1

Distribution of horses reported with traumatic injury to University Veterinary Hospital, Universiti Putra Malaysia according to sex, age, breed and uses from January to December 2023

Signalments	Frequency	Percentage (%)	Confidence Interval (95%)
Sex			
Mare	50	50.5	(0.41, 0.60)
Gelding	46	46.5	(0.37, 0.56)
Stallion	2	2.0	(0.00, 0.05)
Filly	1	1.0	(0.00, 0.03)
Total	99	100.0	
Age			
Foal	1	1.0	(0.00, 0.03)
Adult	77	77.8	(0.70, 0.86)
Geriatric	21	21.2	(0.13, 0.29)
Total	99	100.0	
Breed			
Thoroughbred	35	35.4	(0.26, 0.45)
Warmblood	16	16.2	(0.09, 0.23)
Arabian	11	11.1	(0.05, 0.17)
Polo ponies	32	32.3	(0.23, 0.42)
Criollo	1	1.0	(0.00, 0.03)
Friesian	1	1.0	(0.00, 0.03)
Local pony	1	1.0	(0.00, 0.03)
Unknown	2	2.0	(0.00, 0.05)
Total	99	100.0	
Uses			
Dressage and show jumping	23	23.2	(0.15, 0.32)
Endurance	19	19.2	(0.11, 0.27)
Polo match	27	27.3	(0.18, 0.36)
Pleasure	10	10.1	(0.04, 0.16)
Patrol	6	6.1	(0.01, 0.11)
Unknown	15	15.2	(0.08, 0.22)
Total	99	100.0	

Table 2

Distribution of location of traumatic injury incident took place in horses presented to University Veterinary Hospital, Universiti Putra Malaysia from January to December 2023

Location	Frequency	Percentage (%)	Confidence Interval (95%)
Stable or stall	28	26.2	(0.18, 0.34)
Competition	20	18.7	(0.11, 0.26)
Ridden	20	18.7	(0.11, 0.26)
Paddock	9	8.4	(0.03, 0.14)
Transportation	2	1.9	(0.00, 0.10)
Not mentioned	28	26.2	(0.18, 0.34)
Total	107	100.0	

Note. Out of a total of 99 horses, 8 horses sustained two traumatic injuries, while the remaining 91 horses sustained a single traumatic injury

Table 3

Distribution of the causes of traumatic injury in horses presented to University Veterinary Hospital, Universiti Putra Malaysia from January to December 2023

Causes of traumatic injury	Frequency	Percentage (%)	Confidence Interval (95%)
Kick	12	11.2	(0.05, 0.17)
Stumble/ Fall	11	10.3	(0.05, 0.16)
Improper bit/ saddle	7	6.5	(0.02, 0.11)
Penetration by a sharp object	12	11.2	(0.05, 0.17)
Crash and hit during polo game	9	8.4	(0.03, 0.14)
Transportation	2	1.9	(0.00, 0.10)
Self-inflicted	7	6.5	(0.02, 0.11)
Not mentioned	47	43.9	(0.35, 0.53)
Total	107	100.0	

Note. Out of a total of 99 horses, 8 horses sustained two traumatic injuries, while the remaining 91 horses sustained a single traumatic injury from nail penetration of the sole or other penetration of muscle by sharp objects. Infrequently, traumatic incidents occurred without visible physical injury, although horses exhibited signs of inflammation and recumbency. The occurrences of injuries on various body parts showed significant variation as demonstrated in Table 5. The proportion of injuries on the limb was the highest, in which the injury to the forelimb was significantly more than those that occurred to the hindlimb. Injury to the head region was lesser than the limb, which was attributed to the eye, mouth, ear, and nose, respectively. The traumatic injuries also inflicted the hoof, trunk, chest, and hindquarters. The genital and neck regions exhibited the lowest frequency of distribution.

Table 4

Proportion of traumatic injuries by type in horses presented to University Veterinary Hospital, Universiti Putra Malaysia from January to December 2023

Type of traumatic injury	Frequency	Percentage (%)	Confidence Interval (95%)
Single wound	44	41.1	(0.32, 0.50)
Multiple wounds	21	19.6	(0.12, 0.27)
Fracture	10	9.3	(0.04, 0.15)
Ligament/ tendon injury	16	15.0	(0.08, 0.22)
Eye trauma	9	8.4	(0.03, 0.14)
Hoof and muscle penetration/ puncture	5	4.7	(0.01, 0.09)
No visible injury*	2	1.9	(0.00, 0.10)
Total	107	100.0	

Note. Out of a total of 99 horses, 8 horses sustained two traumatic injuries, while the remaining 91 horses sustained a single traumatic injury. * = Horses were reported to have experienced traumatic incidents exhibited signs of inflammation and recumbency, with an absence of visible external or internal injuries

Table 5

Distribution of the traumatic injuries relative to anatomical location in horses presented to University Veterinary Hospital, Universiti Putra Malaysia from January to December 2023

Anatomical location distribution	Frequency	Percentage (%)	Confidence Interval (95%)
Head			
Eyes	9	7.4	(0.03, 0.12)
Mouth	7	5.8	(0.02, 0.10)
Ear	1	0.8	(0.00, 0.03)
Nose	1	0.8	(0.00, 0.03)
Neck	1	0.8	(0.00, 0.03)

Table 5 (continue)

Anatomical location distribution	Frequency	Percentage (%)	Confidence Interval (95%)
Chest	3	2.5	(0.00, 0.05)
Trunk	6	5.0	(0.01, 0.09)
Hindquarters	3	2.5	(0.00, 0.05)
Genital	1	0.8	(0.00, 0.03)
Forelimb	47	38.8	(0.30, 0.47)
Hindlimb	36	29.8	(0.21, 0.38)
Hoof	6	5.0	(0.01, 0.09)
Total	121	100.0	

Note. The total figure indicates to horses that have experienced one or two injuries, and also horses with multiple wounds presented with injuries at multiple body locations

DISCUSSION

This retrospective study presents a comprehensive description of the frequency and patterns of injuries experienced by a subpopulation of horses in Peninsular Malaysia. Overall, all cases reported to the veterinary teaching hospital within 12-month period showed the occurrence of horses with traumatic injuries was 23.0%. The figure is lower compared to the 40.0% reported by a previous study in the United Kingdom (Owen et al., 2011). This difference could be attributed to the data collection approach used, as this study collected information on the injury incidents reported to the hospital and which were attended by the veterinarian. Meanwhile previous study by Owen et al. (2011) used a questionnaire survey from horse owners who may have reported minor injuries that may not have been attended by a veterinarian. Additionally, a study by Satessa and Lema (2014) reported an overall injury occurrence of 45.5% in working equines, which is higher than the occurrence observed in this study. The nature of these animals as working equines may increase their exposure to injury due to the physical demands and environmental factors associated with their labor. However, our occurrence appeared to agree with the survey studies in Australia and New Zealand that reported their occurrence as 22.5% and 25.0% respectively (Sole et al., 2015; Theoret et al., 2015). Indeed, our figure may underestimate the true prevalence of traumatic injury in Malaysia as it only evaluated a small portion of the horse population

in Peninsular Malaysia. Besides, there is also the possibility that the horse owners did not report the trauma incidents to the veterinarian as well as they treated the condition without veterinary assistance. Similar to situation in Australia, where horse owners are known to manage some wounds independently, which could lead to underreporting of injury cases (Sole et al., 2015). While these studies did not assess the welfare impact of this practice, it raises concerns about the effects on horse health, particularly given the need for proper veterinary care for serious injuries. Moreover, the age and sex distribution exhibited no significant influence on the occurrence of traumatic injury in the horses. However, the breed of horse and its use appear to have an impact on raising the risk of exposure to a traumatic incident. In this study, horses engaged in competitive activities experienced a higher frequency of sustaining injuries. The endurance competition mostly featured by Thoroughbred and Arabian horses, while polo matches involved Polo ponies, and Warmbloods were utilized in dressage and show jumping events. As endurance is considered a strenuous exercise, it is noteworthy that horses were exposed to sharp object penetration on the track, along with falls or stumbles due to fatigue. The speed, inexperienced rider, previous injuries of the horses, track and other unmeasured factors may also co-influence the risk for traumatic injury during an endurance race (Nagy et al., 2013). Moreover, given the nature of the polo that requires the ponies to sprint at maximum speed, turn abruptly, make sudden stops, retain balance when riders shift their weight significantly off-centre, and even collide with other horses, the potential for injuries is consistently high for the ponies as well as for the players. This discovery aligns with the findings of a previous study which also identified tendon injuries, wounds, and splint bone fractures as the most often reported injuries in polo ponies (Inness & Morgan, 2014). Additionally, a significant number of show jumping and dressage horses also encounter difficulties related to their ligaments and tendons. This supports previously described clinical findings that demonstrated a high risk of forelimb superficial digital flexor tendon (SDFT) injury and distal deep digital flexor tendon (DDFT) injury in show jumping horses and hindlimb suspensory ligament injury in dressage horses (Murray et al., 2006; Tranquille et al., 2024). This could be attributed to the repetitive loading during jumping and considerable strain on landing, which relatively cause a great strain on the show jumping horse (Murray et al., 2006; Sousa et al., 2017). Meanwhile, the training regime and surface-related factors normally predisposed the dressage horse to this injury (Murray et al., 2006; 2010). Hence, performance horses are at greater risk of sustaining traumatic injury compared to general-purpose horses. Despite the high number of horses used for sport, many horses were injured while in the stable compared to during competition, where the incident took place at the stable compound or in the individual stalls. Other than mechanical injury, the individual housing practice possibly induces abnormal behaviour in the horses which leads to self-inflicted injury. This study discovered that some horses had self-mutilation behaviours, in which there was a tendency to bite their bodies and as well as to scratch the stall wall. This self-inflicted injury can be caused by firstly normal behavioural response to continuous or intermittent

physical discomfort, secondly by self-directed intermale aggression, or thirdly by stereotypic activity, for example nipping at various areas of the body in a relatively invariant pattern, stomping, or kicking rhythmically against an object (McDonnell, 2008; Winkskill et al., 1995). Additionally, horses that were socially confined also may be injured or injure other horses when they are released to stay in the group, particularly in the paddock (Knubben et al., 2008). These horses were predisposed to demonstrating abnormal behaviours to redirect sexual behaviour, as a defensive mechanism, or to establish hierarchy within a group (Knubben et al., 2008; McGreevy, 2004). These abnormal or exaggerated normal behaviours may be likely to increase the risk of injury to horses within the group. Furthermore, the horse also was injured when ridden, both for training and leisure. One of the issues was caused by poor saddle or bit used. Poorly fitted saddle will cause wounds, particularly at the wither and loin region. An ill-fitting saddle can hinder the horse's thoracolumbar function, leading to stiffness in that area, which may contribute to back pain for the rider (Dyson et al., 2015). Similarly, a saddle that does not fit the rider properly can affect their position and balance, resulting in back or hip pain. Identification of an ill-fitting saddle involves inspecting it both off and on the horse without and with the rider, while observing horse movement. Examine for uneven flocking, ensure the gullet is appropriately sized, and check for complete tree coverage to avoid pressure on the spine. A saddle that tips forward or backward or has uneven panel contact can cause pain and restrict the horse's movement. Indeed, correct saddle fit for horse and rider is an important equine welfare issue. Besides, the bits used might also wound the oral region. The bit induces pressure on the sensitive structures of the horse mouth, which include the lip commissures, buccal mucosa, tongue, and the bars of the lower jaw, and in certain bit types, it also causes pressure to the hard palate and the base of the second premolars (Anttila et al., 2022; Manfredi et al., 2005a; 2005b; 2009). However, an ill-fitting bit can potentially put excessive pressure, pinching or rubbing of oral tissues or limit the movement of the tongue, which eventually causes oral trauma and thus pain for the horse (Björnsdóttir et al., 2014; Mata et al., 2015; Tuomola et al., 2019). Hence, a significant number of traumatic injuries also happen in stables and during ridden due to factors like poor housing practices and ill-fitting equipment, thus it is vital to monitor and understand horses' behaviour as well as to ensure the proper choice of saddle and bits to minimize the traumatic injury incidents. In addition, the cause of most injuries was not determined as it was not mentioned in the patient record as well as owners were not present at the time of injury. However, in this study, kicking by another horse and penetration by a sharp object were the most common mechanisms of injury to happen. There were significantly larger proportion of Thoroughbreds were victims of kick injuries than were other breeds, which is a finding in line with a previous study that revealed Thoroughbreds and Thoroughbred crossbreds as more likely to be victims of kick wounds (Schroeder et al., 2013). This study also found that kick injuries most take place when a group of horses are let into the paddock together, this is a similar scenario to a previous study that demonstrated that kick incidents occurred on pasture (Derungs et al., 2004). As an important

aspect of equine behaviour, kicking serves as a defensive mechanism and a means of establishing group hierarchy, particularly occurring when dominant or socially confined horses are placed together (Knubben et al., 2008; McGreevy, 2004). Penetration by sharp objects occurred mostly at the sole of the hoof, and caused laceration of the corneal of the eye. The nails including the horseshoe nail were the main cause of the punctured sole. Meanwhile, laceration of the corneal normally resulted from stable doors, fence posts, trees, and sand. Both penetration of the sole and corneal by a sharp object can be deadly if it is involved with vital structure and normally is associated with the introduction of pathogenic microorganisms (Burba, 2013; Lazareva et al., 2022). Stumble and fall took place during training or competition, and even at stable. This is contributed by the uneven terrain or slippery surfaces that make horses difficult to maintain their footing. Horses undergo strenuous also may experience fatigue or limb and hoof issues which lead them to a lack of coordination and increased risk of stumbling. Moreover, the traumatic injury also occurred due to the crash and hit during a polo match, which focused more on the polo horses. Despite, the high number of wound cases determined in this study, polo horses are always at risk for tendon injuries due to their nature and need for speed, agility and constant exposure to stress during the match (Inness & Morgan, 2014; Schumacher & Gehlen, 2024). Transportation-associated injuries usually occurred during vehicle movement, and less frequently during unloading, loading and while stationary, in relation to behaviours such as scrambling and panicking (Riley et al., 2016). It has also been reported that mechanical failure of a trailer or truck, driver errors, as well as traffic and road condition contribute to injury during the transportation (Riley et al., 2016). The forelimb and hindlimb of horses experienced the highest number of injuries, with the distal portion of the limb being the most commonly affected area. Wounds were the most prevalent type of injury, followed by problems with ligament and tendon and splint bone fracture. Among the causes of wounds to the limb were related to the penetration by sharp objects, stumbles and falls, kicks as well the injury during polo matches. The lower limb is subjected to injury due to its limited soft tissue, thus resulting in inadequate protection compared to the upper limb and trunk (Kayode, 2017). Wounds that involve the distal aspect of the limb are frequently more problematic due to their closeness to the ground makes them more likely to become contaminated and infected (Jørgensen et al., 2021; Kayode, 2017). Moreover, the head region is also exposed to injury, particularly the muzzle and eye. As mentioned above, the high incidence of injury at the muzzle or generally oral region is normally correlate with the poor choice of bits. It is vital to identify the correct bit size in accordance with each horse's oral dimension. Since, the oral dimensions vary by age, sex, and breed, measuring oral dimensions as part of routine dental examination aids in choosing a bit mouthpiece size that fits to avoid discomfort (Anttila et al., 2022). There is a notable correlation between the eye injuries sustained with performance horses which may be related to penetrating objects such as sand for racehorses and polo sticks in polo ponies, however, there was also exposure of potentially hazardous in the stable, such as feed troughs, water buckets, and hay racks. A study showed

that horses kept in stable, sustained more ocular disease compared to the horses kept primarily at pasture (Ludwig et al., 2025). Even though this study reported more findings on ulcerative eye lesions, the horses are also always at risk to sustain the non-ulcerative eye lesions that able to lead to detrimental conditions such as fungal stromal abscess formation, cataracts and corneal perforations

CONCLUSION

In conclusion, this present study has demonstrated that there is significant occurrence of traumatic injury in the subpopulation of horses in Peninsular Malaysia. It was also shown that the majority of injuries were noted in the horses that participated in competition, however general-purpose horses are also predisposed to traumatic injuries, and the most frequent causes of injury were being kicked by another horse and being penetrated by a sharp object. Most of the cases were presented with wounds and the forelimb and hindlimb were the body parts implicated by most traumatic injuries, which were expensive and time-consuming to treat and may lead to reduced performance, retirement, or euthanasia. Therefore, these findings highlight the need for a better preventive measure, improved management practices, and stronger safety protocols. Ultimately, a comprehensive understanding of these injury patterns will promote the overall health and welfare of horses. This proactive approach can significantly reduce the incidence of traumatic injuries in equine populations.

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First Report and Yield Reduction of Emerging Yellow Spot Disease on Melon (*Cucumis melo*) Caused by Melon Yellow Spot Virus (MYSV) in Indonesia

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ABSTRACT

Typical symptoms of melon yellow spot disease include leaf curling, mosaic, chlorotic spots, fruit discoloration, and cracking, which are constantly found in melon greenhouses in Indonesia. These symptoms lead to considerable losses, reducing fruit weight by up to 66.67% and lowering the Brix score, making the fruit unmarketable. RT-PCR targeting the N-gene of MYSV showed all samples were positively infected. Bioinformatics analysis revealed that Asian isolates of MYSV are highly identical and share a common ancestor, highlighting MYSV as an emerging disease to melon production across Asia.

Keywords: Emerging disease, melon, MYSV, RT-PCR, yield reduction

INTRODUCTION

Melon (*Cucumis melo*) is one of the important cucurbit crops in the world. About 70% of melon productions are in Asia. Indonesia is one of the melon producers in Asia with annual production reaching up to 118,696 tonnes (BPS-Statistics Indonesia, 2022). In the last three years, melon production in Indonesia has decreased by more than 14% due to the high prevalence of plant diseases caused by various pathogens. Viruses account for the most anticipated pathogens for melon due to their rapid transmission and significant losses of up to 100%. There are several virus genera known to be associated with melon diseases in Indonesia, namely Potyvirus, Tobamovirus, Cucumovirus, Begomovirus, Comovirus, and Orthospovirus (AdachiFukunaga et al., 2020; McLeish et al., 2022). Yellow spot disease is currently emerging in Asia and is becoming a major threat to melon cultivation. It is caused by melon yellow spot virus (MYSV) (Chen et al., 2010). MYSV is a member of the Orthospovirus genus. It was known persistently transmitted by thrips and was first reported in Japan (Adachi-Fukunaga et al., 2020; Chakraborty et al., 2018; Kato et al., 2000). Later, MYSV was found in several countries including China (Gu et al., 2012; Sun et al., 2020), Taiwan (Peng et al., 2011), Thailand (Chiemsoombat et al., 2008; Supakitthanakorn et al., 2018), Ecuador (Quito-Avila et al., 2014), and India (Pradeep et al., 2024). The symptoms of MYSV infection are chlorotic spot, mosaic, leaf curl, fruit discoloration, and fruit cracking resulting in production failure and total economic loss due to unmarketable products. Compared to established viral infections like Begomovirus, Orthospovirus infections have the potential to become epidemics in the future. This is due to the wide host range, high genetic diversity, persistent transmission

by thrips, and the lack of resistant sources (Pradeep et al., 2024). During the 2021-2023 survey in several melon production greenhouses in Indonesia, we constantly found melon plants exhibiting symptoms including leaf curling, leaf mosaic, chlorotic spots, necrotic spots, fruit discoloration, and fruit cracking with incidence reaching up to 90% in melon population (Figure 1). These symptoms appear simultaneously with the high thrips (*Thrips parvispinus*) population, resembling typical orthotospovirus infections. These symptoms followed by a high thrips population have never been found before in Indonesia. Considering the significant importance of the disease for melon cultivation and developing management strategies, this research aimed to determine the causal agent of the disease and evaluate the damage to melon cultivation. We carried out molecular detection targeting several common viruses on melon including Tobamovirus, Potyvirus, Begomovirus, and MYSV. However, of all tests carried out, only MYSV detection showed positive results, indicating that the yellow spot disease on melon is caused by MYSV. This finding suggests that yellow spot disease caused by MYSV is a novel pathogen in Indonesia and poses a serious threat to melon cultivation.

MATERIALS AND METHOD

Sample Collection

Melon leaves showing yellow spot symptoms were collected from four greenhouse locations in Central Java: Solo, Bergas, Yogyakarta, and Tegal. Leaves of the diseased plants were collected in purposive sampling. Samples collected from the plant showed leaf mosaic, leaf spot, leaf chlorosis, and leaf necrotic spot in the early generative stage. The early generative stage is the critical time of plants from thrips infestation. Samples were documented, stored dry, and subsequently used for RT-PCR.

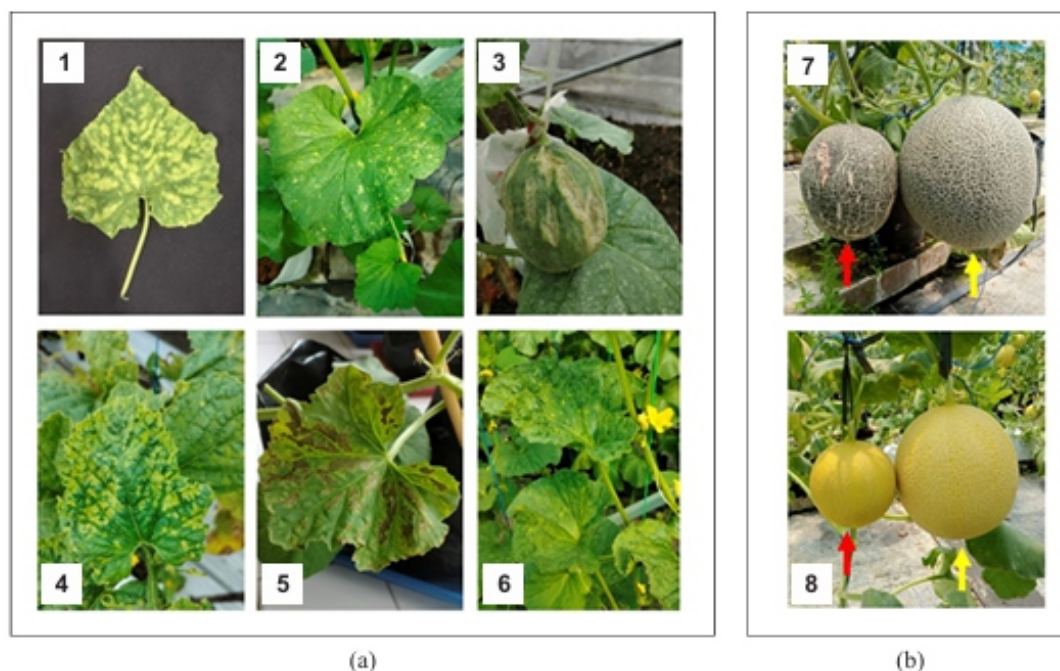


Figure 1. (a) Symptom variations of MYSV in melon plants; and (b) size comparison between fruits. Symptom variations could be a leaf mosaic (1), yellow spot (2), fruit cracking (3), leaf curling and mosaic (4), leaf necrotic (5), or leaf cupping (6). Fruit from diseased plants (7 & 8 red arrows) are smaller, cracked, and might have uneven nets compared to the healthy one (7 & 8 yellow arrow)

Field Observation and Damage Evaluation

Two greenhouses located in Solo and Bergas were utilized to model yield losses in melon due to disease. Each greenhouse housed an estimated 3,000-4,000 plants. Disease impact was assessed by evaluating several parameters: disease incidence, severity, fruit weight loss, and fruit Brix. Disease incidence was determined by calculating the proportion of diseased plants within the total plant population in each greenhouse. Disease severity was assessed by quantifying the number of symptomatic leaves on each plant relative to the total number of leaves. Fruit weight loss was determined by comparing the weight of fruits from diseased plants to those from healthy plants. Finally, fruit Brix was measured using a refractometer.

RNA Extraction and RT-PCR

Samples were subjected to RT-PCR. Total RNA extraction was performed using the Total RNA Mini Kit (Geneaid, Taiwan) according to the manufacturer's instructions. cDNA was performed using a Revertra-ace cDNA synthesis kit (Toyobo, Japan) with an n-hexamer primer. cDNA was subsequently used as a PCR template. PCR was performed in a total 50 µL reaction consisting of 25 µL MyTaq HS Red Mix 2X (Meridian, USA), 2 µL each forward and reverse primers (10 pmol/µL), 19 µL nuclease-free water, and 2 µL cDNA (10 ng/µL) as template. The primers used to target the nucleocapsid (N) gene of MYSV (Charlarmroj et al., 2017). The PCR conditions were pre-denaturation at 95°C for 3 minutes followed by 35 cycles of denaturation at 95°C for 1 minute, annealing at 55°C for 30 seconds, extension at 72°C for 1 minute, and final extension at 72°C for 10 minutes. PCR products were visualized by 1% agarose electrophoresis and subjected to bidirectional Sanger sequencing.

Bioinformatics Analysis

Nucleotide sequence data was analysed using BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi/>) and subsequently deposited in Genbank (Accession No. OR405986-OR405989). Nucleotide alignment was performed using ClustalW. The phylogenetic tree was constructed using MEGA 7 with Neighbor-Joining Method and 1000 bootstraps replication (Kumar et al., 2016).

RESULTS

Field observations revealed characteristic MYSV symptoms including leaf cupping, dark green spots on leaves, and vein yellowing. These symptoms specifically appeared during the early generative phase, from the onset of flowering until fruit set (Figure 1). The early generative phase, particularly during flower formation, is a highly preferred phase for thrips (Ren et al., 2020). The data showed disease incidence can reach up to 85%, indicating that MYSV is prevalent in these locations. Comparison between healthy plants vs diseased plants showed that the disease caused significant weight losses up to 66,67% and significantly lower average Brix score (9 vs 13 on healthy plants) (Table 1). The lower weight and Brix score on diseased plants consequently caused the fruit not to meet the market standard. Moreover, it resulted in total economic losses.

Table 1
Field observation data on two greenhouses

Greenhouses	Population (plants)	Status	Severity (%)	Incidence (%)	Fruit Weight (gr)	Brix
Solo	4000	Healthy	0	0	1500	13
		Diseased	50	85	500	9
		Total loss percentage	0	0	66,67%	30,71%
Bergas	3500	Healthy	0	0	1350	13
		Diseased	35	70	550	9
		Total loss percentage	0	0	59,26%	30,71%

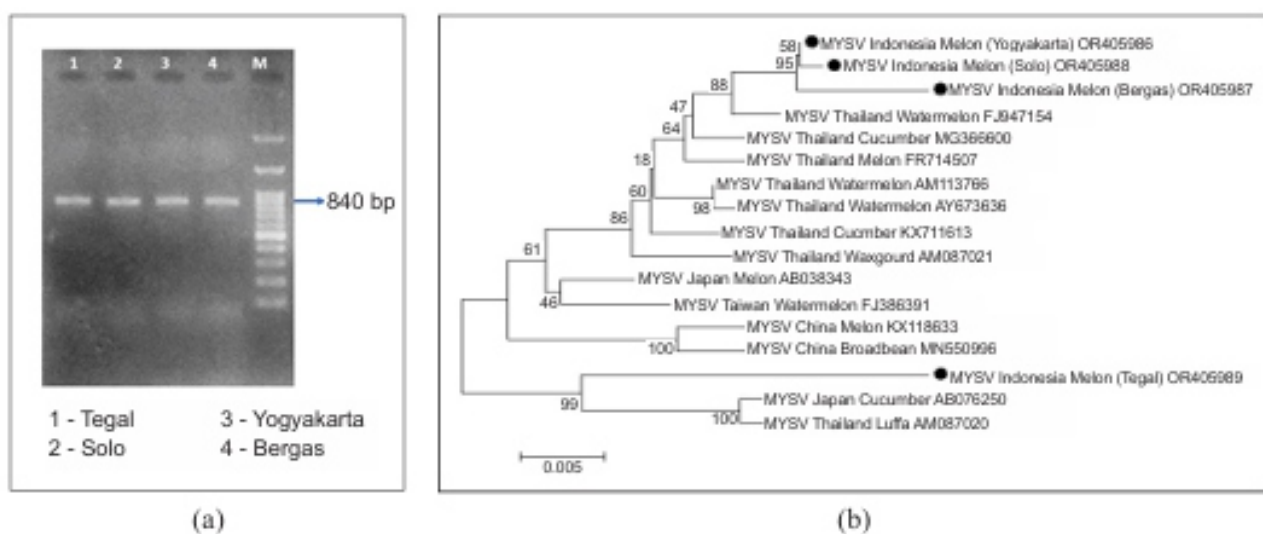


Figure 2. (a) PCR Visualization; and (b) phylogenetic diagram of samples obtained from Tegal (1), Solo (2), Yogyakarta (3), and Bergas (4). The phylogenetic tree of partial N-gene of MYSV using the Neighbor-Joining method with 1000 bootstraps replication showed MYSV isolates in Asia are closely related to each other. All samples tested for MYSV resulted in expected bands of approximately 840 bp (Figure 2). BLASTn search results of nucleotide sequences showed 96-99% identity with MYSV from Japan and Thailand from various host sources. A phylogenetic tree constructed from partial N-gene of MYSV showed Asian isolates are clustered in a single branch indicating these isolates share a common ancestor (Figure 2). These results indicate that the MYSV isolates spread in Asia are closely related. To our knowledge, this is the first report of MYSV in Indonesia.

DISCUSSION

The existence of MYSV in Indonesia poses a serious threat not only to melon cultivation but also to other cucurbitaceae cultivation in general. MYSV was also known to infect several crops including cucumber, watermelon, pumpkin, balsam pear, and chili pepper (Sunpapao, 2012; Supakitthanakorn et al., 2018; Takeuchi et al., 2009). This shows that the potential for disease caused by MYSV in horticultural crops is quite large in the future. Our study indicates that the MYSV detected in Indonesian melons likely shares a common ancestor with MYSV isolates found in other Asian hosts, suggesting potential cross-

-transmission among various plant species, including weeds (Yamasaki et al., 2012). Further distribution of MYSV might exacerbated by common agricultural practices in Indonesia, particularly open-field intercropping. Open-field systems allow the movement of insect vectors between primary crops and weeds, which can serve as alternative virus hosts. While MYSV is primarily transmitted by *Thrips palmi* (melon thrips), it is hypothesized that MYSV might also be transmitted by other thrips species, such as *T. javanicus*, *T. tabaci*, or *T. parvispinus*. *Thrips parvispinus* has become a dominant species in Indonesia, replacing *T. palmi* (Murai et al., 2010; Sartiami & Mound, 2013). The MYSV transmission is exclusively facilitated by thrips in a persistent manner. Thrips is also known as cosmopolitan insects with a broad host range. The widespread presence of thrips and their ability to transmit MYSV across different plant species underscore the need for comprehensive management strategies to control the viral spread. (Chakraborty et al., 2018; Peng et al., 2011). The existence of MYSV as a novel pathogen in Indonesia adds to the long list of diseases in Cucurbitaceae. Thrips as the exclusive vector of MYSV have a short life cycle, reproduce by parthenogenesis, and are quickly resistant to insecticides (Wakil et al., 2023). Up to now, thrips are known resistant to classes of insecticides including organochlorines, organophosphates, carbamates, pyrethroids, and spinosyn (Negash et al., 2020). Until now, there are no known effective chemical pesticides to control thrips (Gao et al., 2012). Meanwhile, control measures using traps, net houses, and biological agents are so far not known to have a significant impact. Therefore, thrips control must be carried out with combined measures in an IPM framework. To effectively control the spread of MYSV, a comprehensive approach is essential, including the eradication of diseased plants, vector management, and greenhouse sanitation. Thrips, as the primary vector of MYSV, play a crucial role in the virus transmission, thus, the control of thrips is the key to disease management. Simultaneously, the eradication of infected plants minimizes the inoculum source, preventing further viral propagation. Implementing strict greenhouse sanitation practices, such as regular cleaning and disinfection, reduces the risk of MYSV transmission by eliminating potential breeding grounds for thrips and other pests. MYSV is expected to emerge as a significant pathogen of cucurbits shortly due to its persistent transmission by thrips and its ability to infect a broad range of host plants. The absence of commercially available melon cultivars with resistance to tospoviruses further underscores the urgent need to develop resistant varieties. This challenge is particularly critical for horticultural breeding programs focused on cucurbits. The insights gained from this research are expected to play a key role in shaping effective MYSV control strategies and guiding the development of tospovirus-resistant melon cultivars, thereby safeguarding future melon production. Further research should focus on the transmission mode and ecological aspect of virus-vector relationships.

CONCLUSION

The occurrence of yellow spot disease in melon caused by MYSV poses a serious threat to melon cultivation in Indonesia. The disease caused significant losses in fruit weight and decreased fruit quality, resulting in unmarketable fruits and total economic loss. To the best of our knowledge, this is the first

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Effects of Eggshells as Organic Fertilizer on Growth of *Brassica juncea* (Mustard Green)

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ABSTRACT

*Eggshell can be very useful as an organic fertilizer in agricultural practices as the main substance found in eggshells are calcium carbonate as well as other micronutrients which might enhance plant growth. The aim of this study was to evaluate the effects of different amounts of eggshells application toward *Brassica juncea* growth. There were 5 treatments with 3 replicates arranged in a completely randomized design (CRD) where treatments are arranged as T1 (control) without eggshell, 1 g of eggshell (T2), 5 g of eggshell (T3), 10 g of eggshell (T4) and 15 g of eggshell (T5) application. Results showed significant differences in crop growth with 11-36% increase in height, 11-70% increase in number of leaves and 26-171% increase in leaves when compared with control, where the most pronounced growth was observed in T5 (15 g of eggshell application). Significant increase was also observed in clay loam soil pH from pH 5.5 to pH 7.52 (T5) and 12-75% increase calcium content in soil but no significant difference was found in plant nutrient contents among treatments. It can be concluded that the addition of eggshells may improve the growth of *B. juncea* as well as soil pH and available calcium content.*

Keywords: *Brassica juncea*, calcium, eggshell, growth, nutrient content, soil pH

INTRODUCTION

Fruits and vegetables are the commodity with high commercial value and have many benefits where vegetables are an important crop dietary component in supplying vitamins and fibres. *Brassica juncea*, from family of Brassicaceae commonly known as mustard green, brown mustard and Chinese mustard is originated from India. This species is a fast-growing vegetable rich in many nutritional compounds including antioxidants, proteins and minerals. Imported leafy vegetables prices specifically *Brassica* are still high even after COVID-19 pandemic had subsided where the price has risen up to 30% and 40% (Tan, 2021) from RM3.00 to RM9.00 due to the escalating costs of agricultural inputs, labour shortage, and irregular weather in Malaysia. Such price increment is also due to the production being lower than its consumption (Federal Agricultural Marketing Authority, 2021) leading to imports of *Brassica* to meet the demand. Hence, increasing the local production while lowering their imports is therefore perceived as a way to alleviate the deficiency of such vegetable supplies. Hence, the high demand for *B. juncea* in the market urges farmers to make extensive attempts in increasing the supply of these vegetables, and generally this is done with higher fertilizer application rates that may be harmful for environment and ecosystem (Najarian et al., 2021). Currently, it is very much recommended to apply organic fertilizers instead of inorganic fertilizers due to the negative impact of inorganic fertilizers (Brankov et al., 2020). Various types of organic waste materials can be used as organic fertilizers such as the undervalued form of household waste, the eggshell (Anugrah et al., 2021). Each day, huge quantities of eggshells are

thrown away as organic waste globally. Eggshell waste disposal contributes to environmental problems where the smell of eggshells provides a site for flies and abrasiveness (Gaonkar & Chakraborty, 2016). As much as 250,000 tons of eggshell wastes are produced annually around the world (Verma et al., 2017). At the same time, the total consumption of eggs by Malaysians is 295 eggs per year, which is the highest rate among Asian countries as eggs are used in many recipes in Malaysia. Most of eggshell consist of calcium carbonate, a common form of calcium while the remaining comprised of proteins as well as other minerals. Moreover, as much as 95-97% of calcium carbonate are in the form of crystals, which are stabilized by the protein matrix inside the eggshell (Haroon et al., 2015). Although most eggshells are made of calcium carbonate, it is assumed that the protein matrix plays an integral role in egg strength. As eggshells contain calcium and other micronutrients, they are the most promising organic source of calcium where 90% of the nutrient is much easier to be absorbed than limestone or other coral sources (Radha & Karthikeyan, 2019). Eggshell contain healthy and balanced calcium attributed to the trace amount of other minerals simultaneously providing an important source of calcium for growing crops while at the same time also able to deter certain pests without the need for chemicals (Karne et al., 2023) since it consists of up to 93% calcium carbonate and other trace elements which make it an excellent source of organic fertilizer (Radha & Karthikeyan, 2019). Henceforth, this research was conducted to study the effects of different amounts of eggshells as an organic fertilizer on growth of *B. juncea* while reducing the organic wastes and promoting sustainable environmental and agricultural practices.

MATERIALS AND METHODS

Preparation of Experimental Materials

The experiment was performed in a greenhouse situated in the Faculty of Plantation and Agrotechnology, Universiti Teknologi MARA, Jasin Campus, Malacca, Malaysia (2°13'44.9"N 102°27'20.8"E). The cultivation of *B. juncea* took about 2 months from October 2023 until November 2023. As much as fifteen (15) healthy seeds of *B. juncea* of uniform in size were sown in a transplanting tray filled with peat moss and watered twice per day. The seedlings were then transplanted with one seedling per polybag when two sets of leaves had developed which was about 14 days after sowing into polybags of size 14 inch × 14 inch. The polybags were filled with topsoil taken at a nearby field in the faculty at 0–15 cm depth of a clay loam texture from Malacca series (Typic Hapludox). As much as 10 kg of topsoil were filled in each of the polybags. The watering was done manually which was provided twice per day in the morning and evening (1 L/ polybag). On the other hand, as much as 3 kg of fresh eggshells were collected, washed, and dried. Then, the eggshells were grinded to a fine powder in a mixer grinder.

Treatment Application

Each seedling was applied with 5 g NPK Green (15:15:15) at every selected period (21 DAS and 30 DAS) while the powdered eggshell treatments were surface-applied on a biweekly basis (8 DAS, 21 DAS, 35 DAS and 50 DAS) for 8 weeks of growth according to the treatments shown in Table 1.

Table 1
Treatments used in the study

Treatment	Description
T1	Without eggshell + 5 g NPK green fertilizer per polybag
T2	1 g eggshell + 5 g NPK green fertilizer per polybag
T3	5 g eggshell + 5 g NPK green fertilizer per polybag
T4	10 g eggshell + 5 g NPK green fertilizer per polybag
T5	15 g eggshell + 5 g NPK green fertilizer per polybag

Experimental Design and Layout

This experiment followed a completely randomized (CRD) design with a total of 15 experimental units consisting of 5 treatments with 3 replications. CRD was chosen as a design for this study since the microclimate in the greenhouse was constant throughout the area where relative humidity was 80%, temperature throughout the greenhouse was 34 °C in the afternoon, sunshine was more than 5.5 hours/day with more than 17 MJ per sq m per day, while mean wind speed was measured at about 10 m/sec.

Data Collection

The plants were grown for 8 weeks (56 DAS) where during harvest, the *B. juncea* plants were measured in terms of plant height, number of leaves, width of leaves, soil pH, as well as soil and plant nutrient analysis. Plant height was measured from the collar of the plant at the surface of the topsoil up to the highest tip of the plant by using a measuring tape while the number of leaves per plant was measured by only counting fully open true leaves. The width of leaves was measured by determining at the widest point perpendicular to the longitudinal axis of the leaf. Soil pH was measured using a pH meter at a 1:2.5 soil-to-solution ratio (Enio et al., 2021). For soil nutrient analysis, available calcium, magnesium, and potassium were determined using ammonium acetate (Nh₄ OAc) (Reeza et al., 2021) while available phosphorus was determined using the method of Bray-2 (Bray & Kurtz, 1945). For plant nutrient analysis, the dry ashing method was employed (Sahrawat et al., 2002).

Statistical Analysis

Analysis of variance (ANOVA) was used to test significant differences between treatments using statistical package for social science (SPSS) (version 21) software while means of the treatments were compared using Tukey's test at $p \leq 0.05$.

RESULTS AND DISCUSSION

Crop Morpho-Physiological Traits

The height of *Brassica juncea* at 56 DAS (at harvest) significantly increased with the increasing amount of eggshell applied, where T5 with 15 g of eggshell was significantly the highest in height with increment of 36% more compared to control (T1) as displayed in Figure 1. Similar results were also found by Anugrah et al. (2021) and Casinillo et al. (2024) where the height significantly increased as the amount of eggshells applied increased. This might be attributed by the high calcium content in eggshells which is known to trigger the establishment of seed and root hairs as well as strengthening the stems resulting in stem elongation produced from repeated cell divisions. Hence, the subsequent elongation of cells produced by the apical and intercalary meristems from shoot apical meristem (Wang & Li, 2008) thus increase the plant height. Therefore, it can be deduced that the application of eggshells may have affect the height of *Brassica juncea*. Similar to the results in plant height, the number of leaves significantly increased as treatments were increased (Figure 2) where T5 had the maximum number of leaves with an increase up to 70% compared to control in T1 (no eggshells) which significantly had the least number of leaves. According to Saragih et al. (2016), the constitution of the eggshell comprises of 97% calcium carbonate, 3% magnesium and 3% phosphorus alongside with traces of sodium, potassium, zinc, manganese, iron, and copper. The added macro and

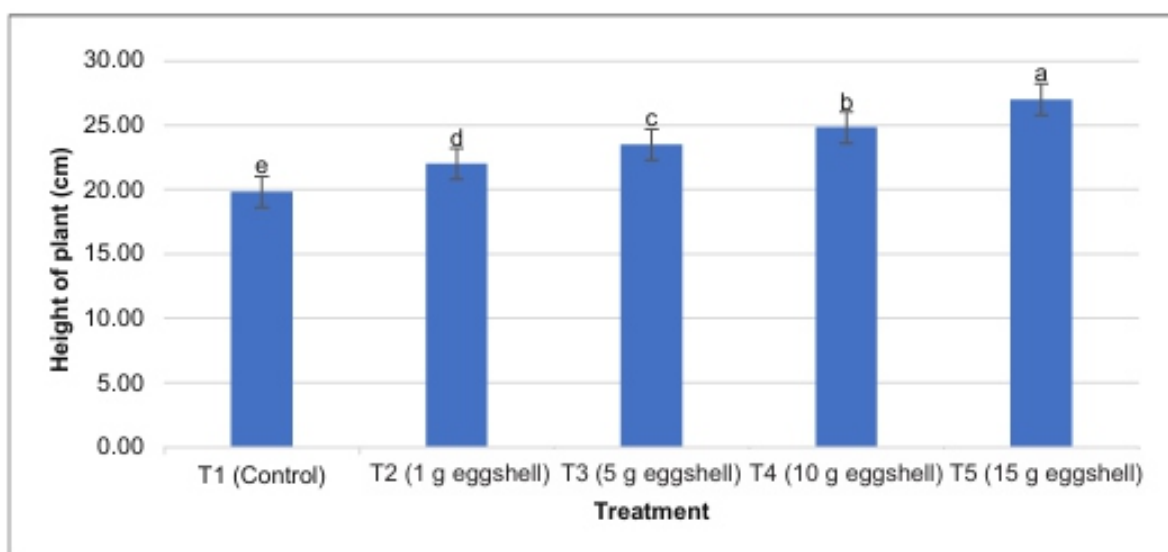


Figure 1. Mean of height of *Brassica juncea* at harvest (56 DAS). Different letters in a column is significantly different according to Tukey's test ($p \leq 0.05$)

micronutrients may have contributed to increasing stem elongation as well as the number of leaves. This result is also consistent with other reports (Muyassir et al., 2022; Radha & Kartikeyan, 2019) whereby higher amounts of eggshells increase the number of leaves in *Brassica juncea* and *Phaseolus vulgaris*. Figure 3 showed that the width of leaves significantly increased with the increasing amount of eggshells applied with increment from 26% (T2) up to 171% in T5. Such observation is similar with the previous parameters of plant height and number of leaves in *Brassica juncea*. The width of leaves for *Brassica juncea* showed that T5 significantly had the highest width while T1 significantly had the lowest width of leaves. Similar finding was also reported by Radha and Kartikeyan (2019) where the leaf area as well as

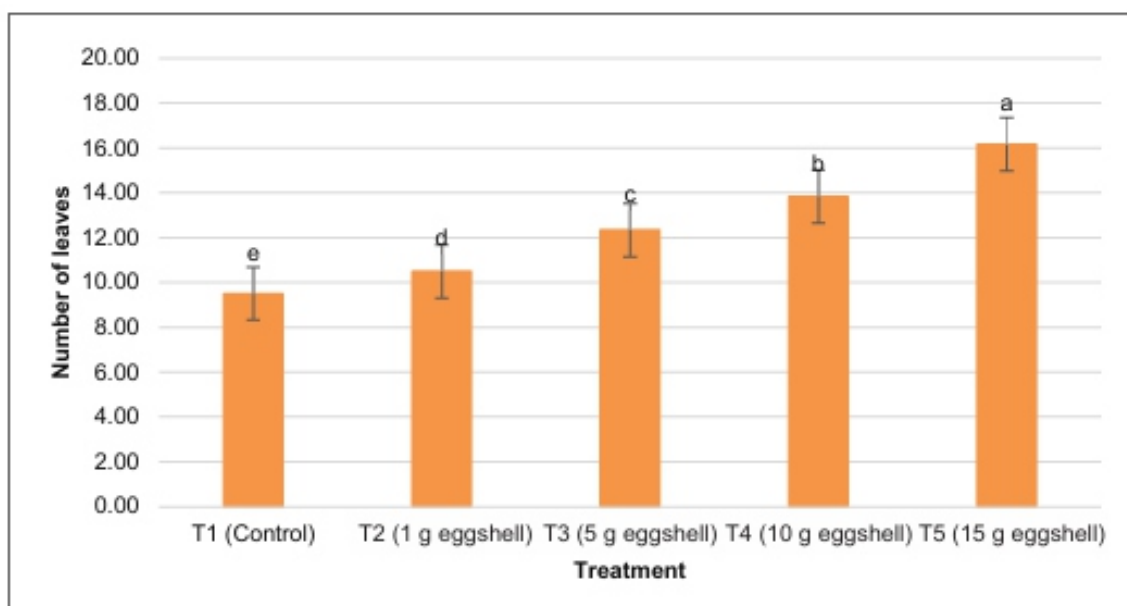


Figure 2. Mean number of leaves in *Brassica juncea* at harvest (56 DAS). Different letters in a column is significantly different according to Tukey's test ($p \leq 0.05$)

chlorophyll content, free amino acid, total protein content and total phenol increased with higher amounts of eggshells applied in cowpea (*Phaseolus vulgaris*). Studies have shown that calcium supports chlorophyll content indirectly by contributing to structural integrity, enzymatic function, and membrane stability, all of which are crucial for chlorophyll production and maintenance (Guo et al., 2023). Calcium is also an essential co-factor for several enzymes involved in the metabolism of amino acids. For instance, enzymes like glutamate dehydrogenase (important for amino acid synthesis) require calcium to function properly (Plaitakis et al., 2017). Adequate calcium levels can enhance the activity of these enzymes, leading to increased synthesis of amino acids, which are building blocks for proteins (Hildebrandt et al., 2015). Calcium is also involved in protein synthesis by regulating the function of ribosomes and other components of the protein synthesis machinery in the cell. It helps stabilize the structure of ribosomal subunits and influences the translation process (Schwarz & Blower, 2016). Therefore, it can be postulated that as calcium is increased, this will help plants improve on structural and enzymatic function, increasing chlorophyll content, producing enzymes important for amino acids formation and thus increasing proteins and leaf size as well. It can be deduced that the application of eggshells may be able to improve the vegetative growth by enhancing the height, number and width of the leaves of vegetables particularly *Brassica juncea*. chlorophyll content, free amino acid, total protein content and total phenol increased with higher amounts of eggshells applied in cowpea (*Phaseolus vulgaris*). Studies have shown that calcium supports chlorophyll content indirectly by contributing to structural integrity, enzymatic function, and membrane stability, all of which are crucial for chlorophyll production and maintenance (Guo et al., 2023). Calcium is also an essential co-factor for several enzymes involved in the metabolism of amino acids. For instance, enzymes like glutamate dehydrogenase (important for amino acid synthesis) require calcium to function properly (Plaitakis et

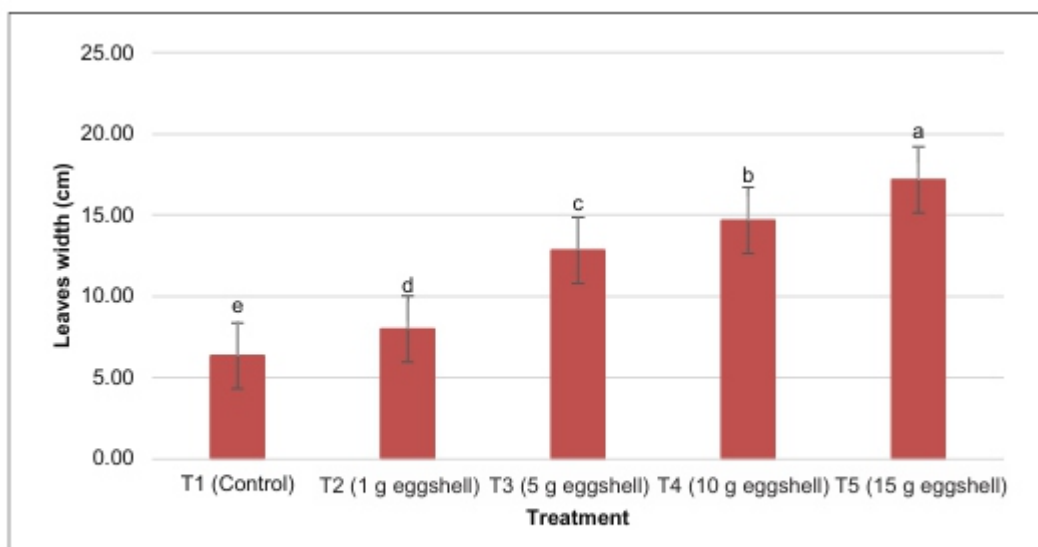


Figure 3. Mean width of leaves in *Brassica juncea* at harvest (56 DAS). Different letters in a column is significantly different according to Tukey's test ($p \leq 0.05$)

al., 2017). Adequate calcium levels can enhance the activity of these enzymes, leading to increased synthesis of amino acids, which are building blocks for proteins (Hildebrandt et al., 2015). Calcium is also involved in protein synthesis by regulating the function of ribosomes and other components of the protein synthesis machinery in the cell. It helps stabilize the structure of ribosomal subunits and influences the translation process (Schwarz & Blower, 2016). Therefore, it can be postulated that as calcium is increased, this will help plants improve on structural and enzymatic function, increasing chlorophyll content, producing enzymes important for amino acids formation and thus increasing proteins and leaf size as well. It can be deduced that the application of eggshells may be able to improve the vegetative growth by enhancing the height, number and width of the leaves of vegetables particularly *Brassica juncea*

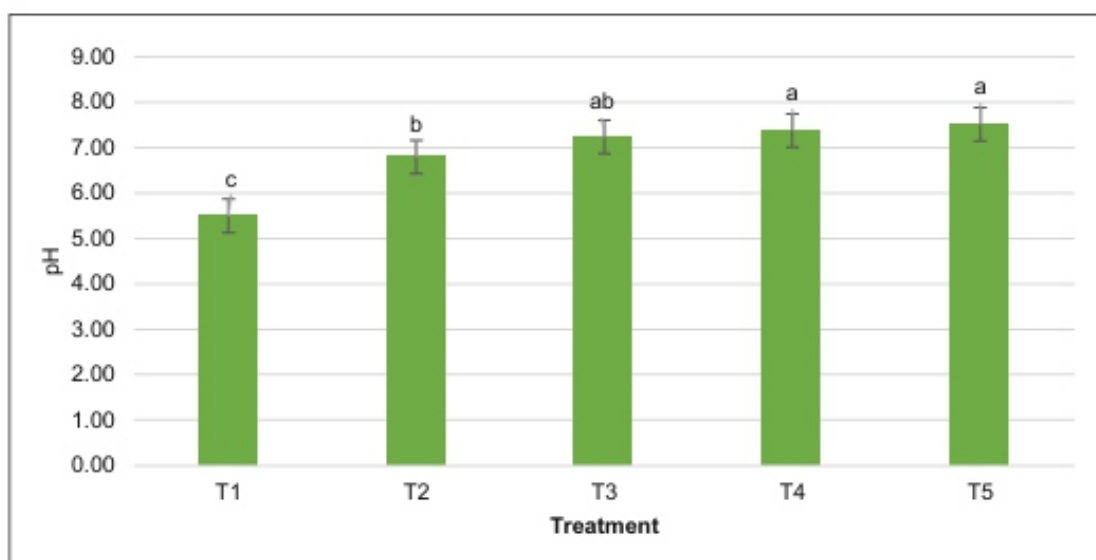


Figure 4. Mean soil pH planted with *Brassica juncea* at harvest (56 DAS). Different letters in a column is significantly different according to Tukey's test ($p \leq 0.05$) for treatments T1 (control), T2 (1 g eggshell), T3 (5 g eggshell), T4 (10 g eggshell), T5 (15 g eggshell)

Soil pH

Significant difference in soil pH was observed in eggshell treatments as compared to control as displayed in Figure 4. The lowest soil pH was recorded at pH 5.5 for T1 whereas T5 recorded pH of 7.52 which was the highest soil pH compared to other treatments. Such increment up to 36% from the initial pH of 5.5 might be attributed to the enrichment of soil with calcium carbonate and others mineral and nutrients presence in eggshell since eggshells contain considerable amount of calcium carbonate (CaCO_3) which can act as an alternative to lime attributed to their high calcium content which able to neutralize the pH of acidic soils (King' Ori, 2011; Wang et al., 2023). It is well-established fact that soil pH of 6.5-7.5 is one of the good characteristics of a fertile soil having optimum physical, chemical and biological properties where all types of crops can be grown. Hence, the use of eggshell was useful in increasing soil pH from an acidic pH to neutral pH.

Soil Nutrient Analysis

The means for soil nutrient analysis particularly phosphorus, potassium, calcium and magnesium are shown in Table 2. It was found that the calcium content was significantly the highest nutrient content, followed by phosphorus, potassium while the least was magnesium. The highest calcium content available in the soil was 3,429 mg kg⁻¹ found in T5 at 15 g of eggshell application. This is to show that the application of eggshells might have contributed to the increase in available calcium in the soil. Such finding is consistent with the report by Vu et al. (2022) whereby application of eggshells onto the soil affected pH and Ca content of the soil, where higher soil Ca was observed in higher eggshell powder rates. It can be deduced that eggshells can be a good alternative source for liming in alleviating soil acidity.

Table 2
Soil nutrient (P, K, Ca and Mg) at harvest (56 DAS)

Treatment	Soil available nutrient (mg kg ⁻¹)			
	P	K	Ca	Mg
T1(control)	814 _a	723.9 _a	1955 _c	432.3 _b
T2 (1 g eggshell)	711.43 _b	517.2 _b	2194 _b	391.4 _c
T3 (5 g eggshell)	559.4 _{cd}	422.67 _c	1875 _c	297.27 _d
T4 (10 g eggshell)	600.63 _c	341.3 _d	1585 _d	251.23 _d
T5 (15 g eggshell)	746.67 _b	550.27 _b	3429 _a	550 _a

Note. Different subscript within a particular nutrient (column) indicate significant difference between treatments

Plant Nutrient Analysis

Table 3 exhibit the mean leaf tissue nutrient analysis between treatments at harvest (56 DAS). Interestingly, only K content in Brassica juncea was found to be significantly different between treatments while no significant differences were found in P, Ca and Mg content in leaf tissue despite the additional increase of calcium content in the soil due to the application of the eggshells. It was also found that K is the highest nutrient content (3.3-4.0%) absorbed by the plant compared to the other

nutrient (0.2-1.6%) where T1 recorded the highest K content in plant tissue (4.084 %) compared to other treatments (3.3-3.5 %). Studies done by Reeza et al. (2023) justified that plants will absorb nutrients that are needed for their growth and not according to the abundance of that particular nutrient in the soil. As such, several reports found that potassium is being highly absorbed by plants compared to other

Table 3
Leaf nutrient content (P, K, Ca and Mg) at harvest (56 DAS)

Treatment	Soil available nutrient (%)			
	P	K	Ca	Mg
T1	0.83 _{ns}	4.084 _a	1.468 _{ns}	0.394 _{ns}
T2	0.8372 _{ns}	3.401 _b	1.527 _{ns}	0.380 _{ns}
T3	0.756 _{ns}	3.405 _b	1.603 _{ns}	0.355 _{ns}
T4	0.6462 _{ns}	3.521 _b	1.469 _{ns}	0.341 _{ns}
T5	0.6016 _{ns}	3.320 _b	1.152 _{ns}	0.270 _{ns}

Note. Different subscript within a particular nutrient (column) indicate significant difference between treatments.
ns= non-significant

nutrients (Tian et al., 2010; Reeza et al., 2023; Vimala et al., 2010). In contrast, magnesium was found to be the lowest nutrient taken up by the plant regardless of the amounts of treatments applied, a similar finding in the aforementioned soil nutrient analysis. Overall, it can be deduced that the application of eggshells had no significant effect on plant nutrient content in Brassica juncea except K content.

Absorption of Calcium from Eggshells into Plant Tissue

The form of Ca in eggshell is in CaCO_3 which is not readily-available for plant uptake whereby plants absorb Ca^{2+} from the soil solution since mass flow and root interception are the principal mechanisms of Ca transport to root surface. According to Ertürk (2020), the CaCO_3 in eggshell needs to be decomposed and converted to Ca which is available prior to plant uptake. This implies that the form of Ca is crucial for plant nutrient uptake. By utilizing crushed eggshells, the calcium content in the soil can be enhanced along with other nutrients found in the eggshell (Faridi & Arabhosseini, 2018; Silveira et al., 2016; Taylor & Locascio, 2004;). Moreover, the benefits of using crushed eggshells is that it requires little energy for preparation, but the drawback is that it requires time for eggshell degradation and decomposition in order to provide nutrients to the plant (Mitchell, 2005; Rai et al., 2014). However, this can be alleviated by introducing liquid and foliar form or tea fertilizer.

CONCLUSION

The study confirmed that different amounts of eggshells application can affect the growth of Brassica juncea (green mustard) by improving plant height, number of leaves and leaves width. The application of eggshells also increased soil pH up to 36% from the initial soil pH and increased soil calcium content. However, it did not affect the majority nutrient content in the plant. Also, eggshell indeed contains macronutrient and micronutrient that are essential for plant growth. Hence, household waste such as eggshells should be used as an organic liming material and organic fertilizer to increase soil pH and the growth of crops. Further research is warranted to explore optimal timing and method of application

eggshells to maximize its benefits in Brassica species.

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Phosphate Solubilizing Bacteria (PSB) and Commercial Rock Phosphate: An Effective Combination for Oil Palm Nursery

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ABSTRACT

*The growth of oil palm trees depends on phosphate availability. Phosphate-solubilizing bacteria (PSB) enhance the availability of phosphorus from phosphate rock by its phosphorus solubilizing ability. Phosphate solubilizing bacteria (PSB) can improve the available phosphorus content provided by phosphate rock through its phosphorus solubilization ability. In the management of palm tree nurseries, it is vital to carefully choose the appropriate type and application rate of phosphate fertilizers based on the growth requirements and soil characteristics of palm saplings to achieve optimal growth outcomes. The purpose of this study was to determine how well oil palm nursery could benefit from the use of commercial rock phosphate in conjunction with phosphate-solubilizing bacteria (PSB). *Bacillus marisflavi* and *Bacillus aryabhattai* populations in 1 g of compost were found to be 2×10^9 and 1×10^8 , respectively, using the plate count method. As seen by leaf count, oil palm size, and frond height, the development rate and quality of oil palm seedlings are greatly enhanced by the combination of Morocco rock phosphate (MRP) and PSB. This study demonstrated that a combination of PSB and commercial phosphate rock is beneficial to oil palm seedlings and sought to determine its efficacy in oil palm nurseries.*

Keywords: *Bacillus aryabhattai*, *Bacillus marisflavi*, commercial rock phosphate, oil palm, phosphate solubilizing bacteria (PSB)

INTRODUCTION

The monoecious oil palm (*Elaeis guineensis* Jacq.) is a member of the Aracaceae family and belongs to the genus *Elaeis* (Corley & Tinker, 2015). Although it is widely grown in tropical nations like Malaysia, Indonesia, Nigeria, and Thailand, it is native to West and Central Africa. The oil palm, a tropical tree, is cultivated for its oil-rich fruit. (Rogers & Kadner, 2022). Fruit oil is a versatile substance that finds application in a range of industries, such as food, cosmetics, and biofuel production. Nearly 40% of all vegetable oil produced is palm oil, making it the most frequently used vegetable oil in the world (Myzabella et al., 2019; Singh et al., 2013). With contributions to global oil palm production and exports of 25.8% and 34.3%, respectively, Malaysia is the second-largest producer and exporter of the crop. The principal importers are from United States, India, the European Union, China and Pakistan (Parveez et al., 2022). Phosphorus is an essential component for oil palm reproductive development, especially for the formation of flowers and fruits. Fruit set, size, and oil content can all be improved with an adequate supply of phosphorus (Etesami, 2019). Only a small amount of the phosphorus in the soil is available to plants, and the majority is unavailable. Phosphate is typically not able to be utilised directly by plants due to its non-bioavailability in soil. Only free, readily available forms of phosphorus can be used by plants from the soil. Soil microbes or plant roots facilitate the availability of soil phosphates (Barroso & Nahas,

2005; Richardson & Simpson, 2011). One of the major non-renewable resources in the world, phosphorus is found in phosphate rock, a type of non-detrital sedimentary rock that is mostly formed in igneous, metamorphic, and sedimentary rocks. In most minerals, phosphorus takes the form of orthophosphate (Ouabid & Raji, 2023). There are several applications for phosphate rock, including the production of phosphate fertilisers, phosphoric acid, pure phosphorus (yellow and red), and other chemical raw materials. The primary ingredient in phosphate fertiliser and phosphate is phosphoric acid (Khan et al., 2022). Phosphate rock deposits are distributed in a somewhat concentrated manner; over 70% of the world's reserves are found in Morocco (Elamrani & Lemtaoui, 2016). The group of rhizobacteria that promote plant growth (PGPR) includes phosphate solubilizing bacteria (PSB). According to Satyaprakash et al. (2017), PSBs have the capacity to solubilize phosphate, changing insoluble phosphatic compounds into soluble forms in soil that are then available for plant absorption. By dissolving and absorbing nonbioavailability P, phosphate solubilizing bacteria (PSB) transform it into bioavailability P to meet plant needs (Chen & Liu, 2019). However, the kind and number of organic acids produced determine the solubilization impact (Tucher et al., 2017). The effectiveness of PSB in improving phosphorus availability depends on various factors, including the type and quantity of organic acids produced. Limited research explored the combined application of commercial rock phosphate and PSB in enhancing phosphorus uptake, growth, and nutrient utilization in oil palm nurseries. This gap highlights the need to investigate the synergistic effects of rock phosphate and PSB on the growth and nutrient dynamics of oil palms, addressing both the immediate agricultural requirements and the broader sustainability challenges associated with phosphorus. This study investigated the effects of commercial rock phosphate and Phosphate Solubilizing Bacteria (PSB) on oil palm growth and nutrient utilisation in oil palm nurseries.

MATERIALS AND METHODS

Production Process of Compost

Various organic materials, such as decanter cake, cocoa shells, and coffee grounds were selected for the composting process. All Cosmos Industries Sdn. Bhd. supplied these organic components, which are commonly used in organic fertilizer production. A compost column was filled with twenty metric tonnes of the different kinds of organic materials that were chosen and mixed well. To speed up the breakdown process, a solution of microorganisms that break down was sprayed onto the thoroughly combined organic materials. The entire composting process took 14 days, with each compost column being stirred once a day by a self-propelled compost turner. Based on the wet weight, 10% of the two PSB bacterial inoculants (1×10^8 -9 cfu/ml) were uniformly sprayed into the compost on day 14

Quality Checking of Compost Samples

We gathered compost samples from All Cosmos Industries Sdn. Bhd. As a result, the generated compost's quality was examined. The moisture content, particle size, pH, carbon to nitrogen ratio, total

N, P, K, Mg, and B content, as well as total organic materials, were all measured for each sample. Using a Shimadzu moisture analyser MOC63u model, the moisture content was determined. The product's moisture content must be less than 8% (Richard et al., 2002). The compost's particle size was measured using a 3.2 mm perforated plate sieve. The acidity or alkalinity of a substance is indicated by its pH value. In a sterile beaker, 5 g of the sample were weighed. The sample was then stirred with a glass rod and 50 mL of distilled water added. The pH metre was calibrated using three buffer solutions with pH values of 4.0, 7.0, and 10.0 prior to pH testing. The material was placed in a suspension of distilled water (5 g/50 ml) and left for two hours to measure its pH. Using the dry oxidation or combustion method and a CHNS analyser, the total organic carbon and total nitrogen were calculated. Samples were crushed, weighed, and freeze-dried before was placed in a tin capsule containing vanadium pentoxide and burned in a reactor at 1000°C. After the sample and container melted together, the tin sped up a strong reaction (flash combustion) in an oxygen-improved environment for a limited period (Ge et al., 2022). Total P, K, Mg, and B contents were determined using the inductively coupled plasma atomic emission spectrometry method in accordance with (ICP-AES). A porcelain crucible containing one gramme of the material was filled, heated to 500°C for two hours, and then removed. After adding 3 mL of HNO₃, the sample was roasted on a hot plate at 100°C until it was dry. After being reinserted into the muffle furnace, the crucible was muffled for a further hour at 500°C. After allowing the crucible to cool, 10 mL of HCl were added. The material was transferred and then diluted in a 50 mL volumetric flask using deionized water. AOAC Method 985.01 analysis was performed on the sample ash solution that was collected (Hemidat et al., 2018). The total organic matter was determined using the loss-on-ignition method (Chai et al., 2013). A 2 g oven-dried sample was weighed and placed it in a silica crucible and heated it to 550°C for 24 hours in a muffle furnace. The weight difference between the initial sample, the burned sample, and the leftover ash was used to quantify the amount of organic matter.

Survival of PSB Cultures in the Compost

To create compost, two PSB cultures were introduced (1×10^8 -9 cfu/ml for each strain). The plate count method was used to determine the total number of PSB in the sample. The term “total viable count” describes the entire number of colonies. The 95 ml of sterile phosphate buffer saline (PBS) was placed within a conical flask, into which 10 g of fertiliser samples were weighed and dissolved. After that, the conical flask was put in a shaking incubator set to 200 rpm for ten to fifteen minutes. Sterile distilled water was used for serial dilution up to a dilution factor of 10⁻⁹. For the purpose of phosphate solubilization bacteria growing media, Pikovskaya agar medium was produced. The glass hockey stick was then used to distribute the suspension across the agar plate surface. After that, plates were incubated at room temperature 27°C for up to 7 days, the colony forming units (CFU) were counted (Chung et al., 2005).

Field Study

The Tenera oil palm of three months old were planted in the field of Ladang 10, Faculty of Agriculture with different treatment (Table 1). The size and shape of the oil palm seedlings chosen as the experimental samples were comparable. A thin layer of soil was placed inside 20 × 20 black polybags housing oil palm seedlings. Then, each group - aside from the control group—received 100 g of the matching therapy (Figure 1). Finally, soils were placed into the polybags (Chao, 2018). Vegetative measures, chlorophyll readings, period yield records, and the phosphorus content of leaves and rachis were all observed and documented as parameters for comparison across all treatments. A total of 120 oil palm seedlings were arranged in a Randomized Complete Block Design (RCBD), with five replications each treatment and six oil palm samples each replication, as shown in Figure 2.

Table 1
Treatment for oil palm nursery

Treatment	Detail of fertilizer application
T1	Control, no fertilizer
T2	Moroccan Rock Phosphate (MRP)
T3	30% Compost + Phosphate Solubilizing Bacteria (PSB) + 70% Moroccan Rock Phosphate (MRP)
T4	Compost + Phosphate Solubilizing Bacteria (PSB)



Figure 1. Oil palm seedlings in black polybags

Data Analysis

Five replicates of each treatment were used in the analysis, which followed the RCBD experimental design. The Prism 9 statistical analysis system and the analysis of repeated measures ANOVA were used to analyse the field data. There were notable variations ($P < 0.05$) between the treatments.

RESULTS

Formulation of Compost

The composition of the compost used in this investigation is presented in Table 2. Analysis of the compost sample revealed that the concentrations of all six heavy metals were below the detection limits of the instrument. Additionally, no *Salmonella*, *Escherichia coli*, or other pathogens were detected in the compost.

Survival Test of Two PSB in Compost

In this study, the PSB inoculant was evenly sprayed on the compost. The results for PSB populations in the compost, which were reconfirmed, are shown in Table 3.

The Effectiveness of Phosphate Solubilizing Bacteria on Oil Palm at the Nursery

Three months after planting, the data was first gathered (3 MAP). Thereafter, planting took place for six months (6 MAP) and eight months (8 MAP). An appendix contains a summary of all the data. Three-month-old oil palm plants showed no discernible variations in the majority of the characteristics. This is

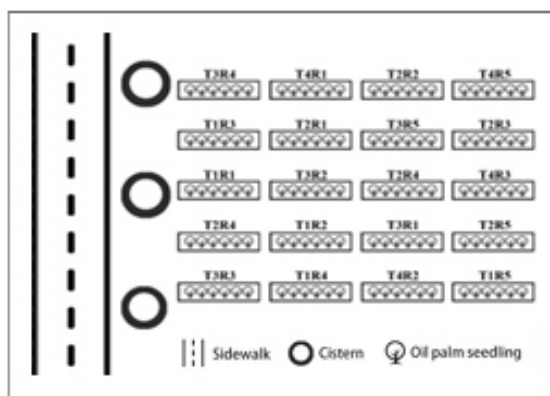


Figure 2. Experimental design

Table 3

PSB population in 1 g of compost

Phosphate Solubilizing Bacteria	CFU/g
<i>Bacillus marisflavi</i>	2×10^9
<i>Bacillus aryabhattai</i>	1×10^8

Table 2

Physicochemical and microbiological analysis of compost

Test Parameter	Unit	Result
pH	-	6.7
Electrical Conductivity	mS/cm	3.16
C/N ratio	-	15.01
Organic Matter	%	66.7
Moisture Content	%	40.1
Total Nitrogen, N	%	2.4
Phosphorus, P	%	0.2
Potassium, K	%	1.1
Calcium, Ca	%	0.6
Magnesium, Mg	%	0.3
Nickel, Ni	ppm	N.D (<0.01)
Chromium, Cr	ppm	N.D (<0.01)
Lead, Pb	ppm	N.D (<0.01)
Cadmium, Cd	ppm	N.D (<0.01)
Arsenic, As	ppm	N.D (<0.01)
Mercury, Hg	ppm	N.D (<0.01)
<i>Escherichia coli</i>	MPN/g	N.D (<0.01)
<i>Salmonella</i>	-	absent

Note. N.D mean not detected

explained by the fact that oil palm seedlings with comparable sizes and shapes were chosen for the experiment. Figure 3 describes the size of the oil palm at three, six, and eight months old in relation to its vegetative growth. When the oil palm was three months old, there were no appreciable differences between the four treatments. Nevertheless, variations in growth are only discernible between the ages of 6 and 8 months. At 6 months, oil palm size in T2, T3, and T4 was significantly greater than in the control group (T1). Notably, T3 outperformed both T2 and T4, suggesting that the treatment with 30% compost + Phosphate Solubilizing Bacteria (PSB) + 70% Moroccan Rock Phosphate (MRP) is more effective in promoting growth. A similar pattern was observed at 8 months, although T2 did not show a significant difference compared to T1. Significant differences were observed when comparing the heights of the first and third fronds in most plot samples, with the exception of the first and third frond heights at three months, as shown in Figures 4 and 5. Regarding the height of the first frond at 6 months, T2, T3, and T4 demonstrated significantly better performance compared to the control group (T1). Interestingly, T3 outperformed T2 and T4, indicating that the treatment with 30% compost + Phosphate Solubilizing Bacteria (PSB) + 70% Moroccan Rock Phosphate (MRP) is more effective in these treatments. Similarly, at 8 months, the same trend was observed, though T2 did not show a significant difference compared to T1. Furthermore, the height of the third frond followed a similar trend in group mean scores

to that of the first frond at both six and eight months. To conclude, the oil palms in the treatment without PSB-mixed compost, aged 6 and 8 months, have shorter fronds compared to those in the treatment with it. Additionally, the treatment with MRP shows a noteworthy distinction from the group without MRP in oil palms of the same ages. Figure 6 shows the results of chlorophyll content in oil palm leaves. At six months, a comparison of the group mean scores revealed that T1, T3, and T4 demonstrated significantly higher efficacy than T2. The chlorophyll content in the 8-month-old oil palm leaves followed the same trend. In summary, the group with only MRP showed lower values compared to the others. Figures 7 and 8 displayed the width and thickness of the third frond data analysis for oil palm. The thickness of the oil palm third frond in both six months and eight months were found to the comparison of mean scores among groups T1, T3, and T4 showed significantly higher efficacy than T2. The six months shows the extreme significant difference which

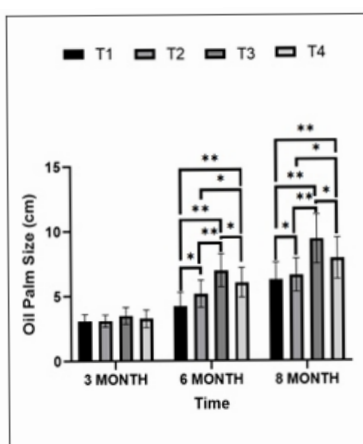


Figure 3. The size of 3 months, 6 months, 8 months old oil palm seedling
Note. Measurement was recorded the 3 months old oil palm, 6 months old oil palm and 8 months old oil palm. The repeated measured ANOVA significant difference all-pairwise comparison test. It was used to detect statistical differences among the means at $P=0.05$ and $P=0.01$ significance level. The bars were connected by zigzag line with * symbol means they show a significant difference (*: $p<0.05$; **: $p<0.01$). The lack of zigzag line represents no significant difference between treatments

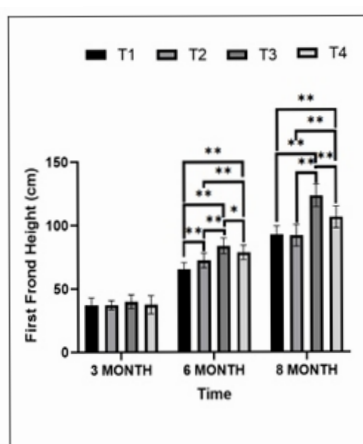


Figure 4. The height of 3 months, 6 months, 8 months old oil palm first frond
Note. Measurement was recorded the 3 months old oil palm, 6 months old oil palm and 8 months old oil palm. The repeated measured ANOVA significant difference all-pairwise comparison test. It was used to detect statistical differences among the means at $P=0.05$ and $P=0.01$ significance level. The bars were connected by zigzag line with * symbol means they show a significant difference (*: $p<0.05$; **: $p<0.01$). The lack of zigzag line represents no significant difference between treatments

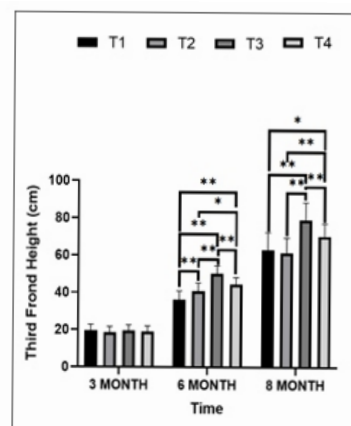


Figure 5. The height of 3 months, 6 months, 8 months old oil palm third frond
Note. Measurement was recorded the 3 months old oil palm, 6 months old oil palm and 8 months old oil palm. The repeated measured ANOVA significant difference all-pairwise comparison test. It was used to detect statistical differences among the means at $P=0.05$ and $P=0.01$ significance level. The bars were connected by zigzag line with * symbol means they show a significant difference (*: $p<0.05$; **: $p<0.01$). The lack of zigzag line represents no significant difference between treatments

the p-value less than 0.01, however the thickness of the eighth-month oil palm third frond only exhibited a p-value less than 0.05. The width of the oil palm's third frond, however, did not provide a noteworthy outcome. The significant increase in frond thickness in treatments with RP and PSB (T1, T3, T4) is indicative of better plant health, enhanced nutrient availability, and overall improved growth, especially at the early stages of development (six months). This the hypothesis that frond thickness is a key indicator of the plant response to fertilizer treatments, which can influence its long-term productivity and oil yield. The lack of significant results for frond width further suggests that thickness is a more reliable measure of plant efficacy in this context. Only after six months did the quantity of oil palm leaf significantly different in Figure 9. The group mean score comparison results with evident differences were the effectiveness of T2, T3, and T4 was significantly greater than that of control group (T1). T3

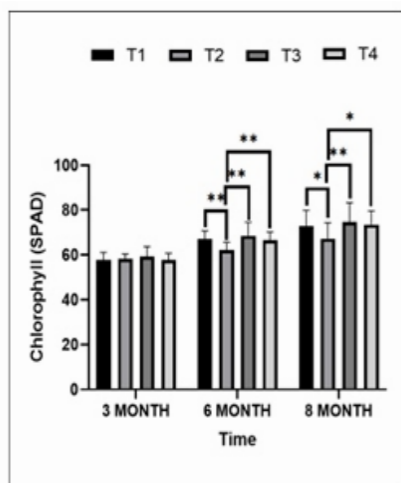


Figure 6. The chlorophyll content in 3 months, 6 months, 8 months old oil palm leaves
Note. Measurement was recorded the 3 months old oil palm, 6 months old oil palm and 8 months old oil palm by SPAD502. The repeated measured ANOVA significant difference all-pairwise comparison test. It was used to detect statistical differences among the means at P=0.05 and P=0.01 significance level. The bars were connected by zigzag line with * symbol means they show a significant difference (*: p<0.05; **: p<0.01). The lack of zigzag line represents no significant difference between treatments

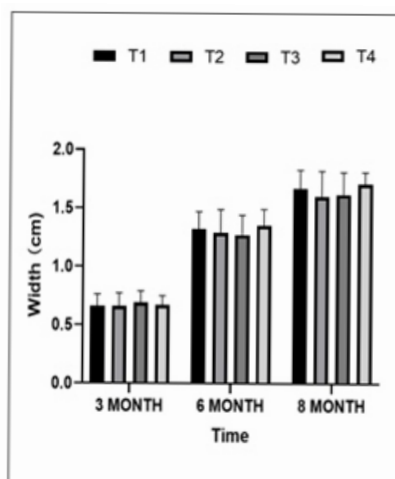


Figure 7. The width of 3 months, 6 months, 8 months old oil palm third frond
Note. Measurement was recorded the 3 months old oil palm, 6 months old oil palm and 8 months old oil palm. The repeated measured ANOVA significant difference all-pairwise comparison test. It was used to detect statistical differences among the means at P=0.05 and P=0.01 significance level. The bars were connected by zigzag line with * symbol means they show a significant difference (*: p<0.05; **: p<0.01). The lack of zigzag line represents no significant difference between treatments

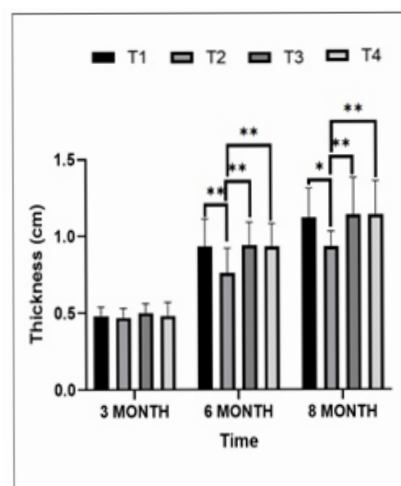


Figure 8. The thickness of 3 months, 6 months, 8 months old oil palm third frond
Note. Measurement was recorded the 3 months old oil palm, 6 months old oil palm and 8 months old oil palm. The repeated measured ANOVA significant difference all-pairwise comparison test. It was used to detect statistical differences among the means at P=0.05 and P=0.01 significance level. The bars were connected by zigzag line with * symbol means they show a significant difference (*: p<0.05; **: p<0.01). The lack of zigzag line represents no significant difference between treatments

which possesses PSB and MRP showed significantly higher efficacy than T2 and T4 which only possess single ingredient. There are observed significant differences among all the treatments in 6 months old oil palm trees. Eight-month-old oil palm trees were divided into rachises and leaves after eight months of growth. After drying, these plant portions were transported to the laboratory for a study of their phosphorus content. The findings of the laboratory analysis are displayed in Figure 10. There were no notable variations found between the other treatment groups, with the exception of the phosphorus content discrepancy between the rachises of T1 and T3. Variations in phosphorus absorption levels could be the cause of the notable differential in phosphorus concentration between T3 and T1 in the rachises.

DISCUSSION

Survival Test of Two PSB in Compost Table 3 shows that there were two PSBs in 1 g of compost, with respective populations of 2×10^9 and 1×10^8 . It demonstrates that two PSBs could live and procreate in compost. This suggests that composting offers the PSBs the perfect habitat for growth and proliferation. Additionally, this research might provide insightful information for managing and optimising composting operations. Bacteria that solubilize phosphates can break down and make use of phosphorus molecules. They have the ability to change organic phosphorus into inorganic forms, increasing compost's phosphorus concentration. This gives compost a higher nutritional content and gives plants the phosphorous minerals they need. Understanding these microbes' functions in their natural habitats and how they interact with other creatures depends on this research (Timofeeva et al., 2022). PSB are essential environmental microbes that have strong ecological flexibility in their natural environments

and a wide range of potential uses in the fields of biological control and environmental restoration. They also aid in the breakdown of organic contaminants, the synthesis of antibiotics, and the decomposition of organic materials (Numan et al., 2018). Compost maturation can be accelerated by modifying the growth and reproduction of phosphate-solubilizing bacteria through manipulation of composting parameters, including temperature, moisture, and ventilation.

Field Data Analysis

The oil palm seedlings treated with MRP and PSB compost in our experiment showed noticeably quicker growth rates and outperformed the control group. Malhotra et al. (2018) claimed the ability of phosphorus to promote early branching and budding, accelerate the growth of plant stems and roots, and enhance seed germination. Oil palms absorb phosphorus most quickly in their seedling stage, therefore a phosphorus deficit at this

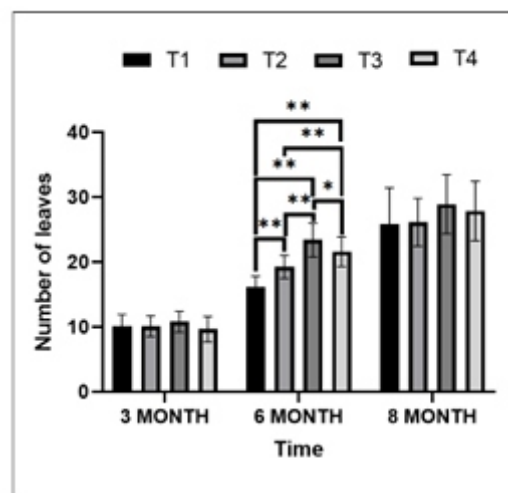


Figure 9. The number of 3 months, 6 months, 8 months old oil palm leaves

Note. Measurement was recorded the 3 months old oil palm, 6 months old oil palm and 8 months old oil palm. The repeated measured ANOVA significant difference all-pairwise comparison test. It was used to detect statistical differences among the means at $P=0.05$ and $P=0.01$ significance level. The bars were connected by zigzag line with * symbol means they show a significant difference (*: $p<0.05$; **: $p<0.01$). The lack of zigzag line represents no significant difference between treatments

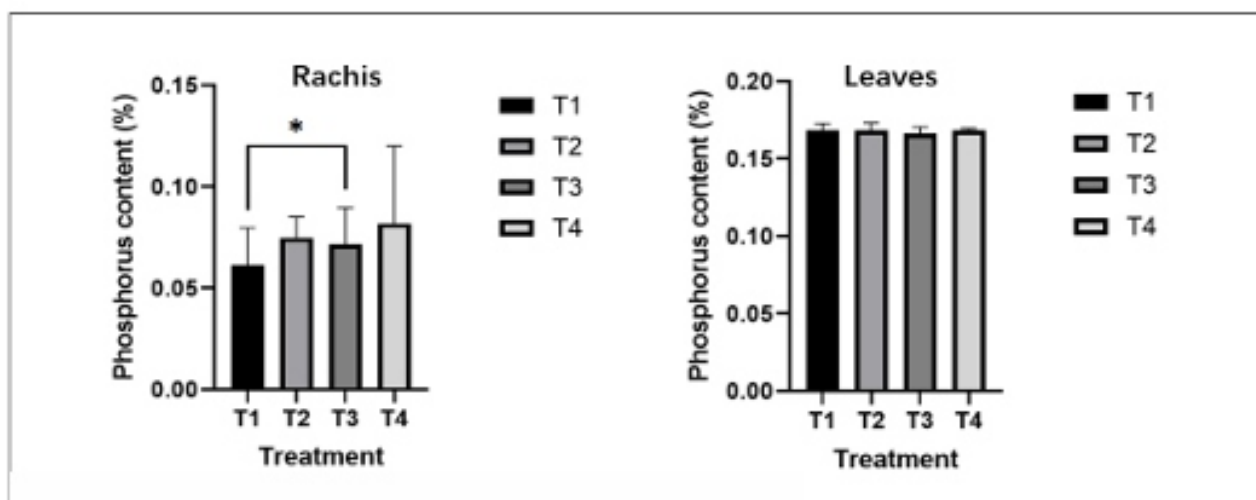


Figure 10. The phosphorus content of 8 months old oil palm rachis and leaves Note. Analysis data was from the dried rachis and leaves of 8 months old oil palm. The repeated measured one-way ANOVA significant difference all-pairwise comparison test. It was used to detect statistical differences among the means at $P=0.05$ significance level. The bars were connected by zigzag line with * symbol means they show a significant difference ($p<0.05$). The lack of zigzag line represents no significant difference between treatments

time can negatively impact the oil palm's ability to expand in the future (Ajeng et al., 2020; Lovelock et al., 2006). According to Abidemi et al. (2006), most seedlings' sizes were considerably enlarged when they were given phosphorus biofertilizer. An essential nutrient for plant growth is phosphorus. Khattab et al. (2019) showed Plants that are poor in phosphorus display signs like stunted growth, short stature, delayed production of flower buds, and increased fruit and blossom drop. Variations in the absorption of

total phosphorus could affect the height of the fronds. The results of Kumar et al. (2020) also demonstrated that seed germination, as well as other plant parameters such as height and weight, significantly increased in plants treated with PSB. Additionally, the frond height of the control group, which includes MRP and PSB at 6 and 8 months, shows a significant difference compared to the other groups. This indicates that the combination of MRP and PSB positively impacts frond height in oil palms. The results also show that treatments with MRP and PSB significantly improved chlorophyll content, frond thickness and highest leaf quantity in oil palm at early stages of development, outperforming other treatments. These findings align with research by Etesami (2019), which highlighted the positive impact of PSB on nutrient availability and plant growth. Plants with phosphorus deficit may develop without branches and shed their leaves early. Heppell et al. (2015) proved older leaves close to the base of the stem exhibit the first signs of phosphorus deficit, which then spread upward. This could help to explain the notable variations in all treatments that were seen in oil palm plants that were six months old. Plants typically contain between 0.1% and 0.5% of phosphorus in their dry weight. The movement of growth centres inside a plant and metabolic activities are intimately linked to the dispersion of phosphorus. Phosphorus is predominantly found in young shoots and root tips during the vegetative growth phase, which contributes to the plant's pronounced apical dominance (Gaiero et al., 2020; Viegas et al., 2021). This could account for why leaves have more phosphorus than rachises. Rachises sustain leaves, flowers, and fruits inside a defined area in addition to acting as a conduit for nutrients and water. Stems can occasionally carry out photosynthesis, store nutrients, and aid in reproduction. As a result, the rachises can show differences in their amounts of phosphorus absorption.

CONCLUSION

By phosphate solubilization, phosphate-solubilizing bacteria increase the amount of phosphorus that is available in the soil. They greatly raise the soil's total phosphorus levels when paired with MRP. Phosphate solubilizing bacteria (PSB) and Morocco rock phosphate (MRP) work in concert to promote oil palm seedling growth, which improves growth rates and quality. In addition, it improves soil conditions and increases oil palms' ability to absorb and utilise phosphorus. As a result, using MRP and PSB together while planting oil palms works well to improve the yield and quality of the oil palm. *Bacillus marsflavi* and *Bacillus aryabhattai* are the two PSB cultures which showed a high rate of survival. The benefits in terms of leaf count, oil palm size, and frond height are enhanced effectively when Morocco rock phosphate (MRP) and phosphate solubilizing bacteria (PSB) are combined. Oil palm seedlings have been shown to benefit from commercial production.

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