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Research Article

Efficacy of selected botanicals, phosphine and boric acid against pulse beetle, *Callosobruchus chinensis* under laboratory conditions

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ABSTRACT
Insect pests are a threat to both growing field crops and stored grain commodities during storage. There are different stored pests which affect the mung bean (*Vigna radiata*) all over the world. Most important destructive pest is Pulse beetle *Callosobruchus chinensis* which cause lot of losses during storage of mung bean. Different pesticides are being used to control the Pulse beetle but these pesticides have harmful effect on human health, grain quality and development of insect resistant population. The replacement approaches for pest management in food items is a growing need in the food sector, which should match customer requests for pesticide reduction or eradication. In the present study impact of phosphine, boric acid and botanicals (Neem and Eucalyptus) on adult stage of pulse beetle *Callosobruchus chinensis* was evaluated at 65 ±5% RH, 30±1°C. The pulse beetles were exposed to phosphine, boric acid at the different concentrations like phosphine 2g, 1g, & 0.5g, boric acid 0.25g, 0.12g & 0.06g and botanicals leaves 2g, 5g and 10g for 24h, 48h and 72h were evaluated. Maximum mortality (98%) by phosphine at 0.25 g, boric acid (95%) at 0.25 g, neem leaves (96%) at 10g and eucalyptus (95%) at 10g after 72h was observed. This technique is the best and eco-friendly to controlling pulse beetles. This technique has no adverse or residual effect on the quality of grains.

Keywords: Botanicals; eco-friendly; phosphine; boric Acid; *Callosobruchus chinensis.*

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INTRODUCTION

Pulses are a significant crop grown for food all over the world, particularly in South Asia (Bangladesh, India, and Pakistan). Compared to other pulses, mung beans are more significant (Nair et al., 2013). The mung bean is an excellent source of fiber, carbs, vital fatty acids, vitamins, minerals, and high-quality, highly digestible proteins (Mubarak, 2005). The entire area in Pakistan planted with mung beans in 2018–19 was 164,000 hectares, with a notable production of 253,000 metric tons and an annual mung bean production of 3.3% (GOP, 2022). Punjab leads the Mung bean production with 87% share, Balochistan 6%, KPK 5% and Sindh 4%. (Anonymous 2016-17) (Haqqani et al., 2000). This pulse is consumed in a variety of dishes, including noodles, bread, cakes, jams, sweets, sprouts, dhal, fried beans, bean paste, and processed grains (Asif et al., 2013) (Nair et al., 2013). The percentage of total grain production lost by storage pests ranged from 5 to 15%, and in cases of severe infestation, such losses could reach 50% (Gabriel and Hundie, 2006; Thompson and Reddy, 2016). During storage, pulses are attacked by more than 150 insect pests.

The bruchids (Callosobruchus spp.) that include *Callosobruchus maculatus* (Fabricius), *C. chinensis* (Linnaeus), *C. analis* (Fabricius), and *C. phaseoli* (Gyllenhal) are among the most detrimental pests (Mishra et al., 2015; Nchimbi-Mosolla and Misangu, 2001). Due to their generalized legume diets and widespread range, the pulse beetle (*C. chinensis*) is one of the most destructive pests of stored legume business, particularly the mung bean industry. Even entire losses could happen over the typical pulse storage period of three to six months (Somta et al., 2006) (Duan et al., 2014) (Swapan, 2016). The life cycle of pulse beetle is varied from 26 to 29 days with an average of 27.0 days. The eggs are found singly and are yellow in color before they hatch, becoming opaque (Kyogoku and Nishida, 2013). In order to prepare for the competition for nutrients in the legume, their eggs become much smaller in high population density areas, resulting in smaller adults and less fit larvae (Yanagi et al., 2013). The grubs have shorter legs and a yellowish-white color. The size of the grub and head capsule casting were used to identify the four instars of *C. chinensis* grubs (Augustine and Balikai, 2019). Pupation takes place inside the legume and the color of pupa is dark brown. The adult grows to a length of roughly 5 mm. The adult stage is characterized as having spots of black and grey coloration all over its body and being brown in color. The abdomen of the female is slightly longer than the elytra, and it is white in colour with two oval black spots on it. This species exhibits some sexual dimorphism, with the female being larger and heavier than the male beetle. Males have pectinate antennae, while females have serrate antennae (Varma and Anandhi, 2010). Through the creation of a tiny hole, the adult and grub consume the grain. White eggs on the seed's surface and spherical exit pores with the seed coat's "flap" can both be used to identify infested stored seed. During storage, the pulse beetles cause a loss of pulses of between 5 and 10 percent (Ngamo et al., 2007). The seed loses its capacity to germinate and its nutritional value when infested by this insect (Sharma, 1984). At beginning in the field the pulses are infested and continuing all the way to storage. The months of July through October have been identified as having the most product damage (Yewle et al., 2022). The pulse beetle not only degrades the quality of the grains but also reduces their nutritional value, rendering them unfit for human consumption and unfit for commerce (Yaseen et al., 2019). Despite their demonstrated efficacy in a wide range of application scenarios, methyl bromide. However, their biggest challenge today is the emergence of resistance in almost all types of stored grain insect populations globally (Nayak and Daglish, 2018). The use of methyl bromide as a fumigant has been prohibited. Because it reduces ozone levels in the atmosphere (Rajendran and Sriranjini, 2007). The additional fumigants employed to control *Callosobruchus maculatus* (Fabricius), *C. chinensis* (Linnaeus), *C. analis* (Fabricius), and *C. phaseoli* (Gyllenhal) are carbonyl sulphide, ethyl formate, hydrogen cyanide, methyl iodide, propylene oxide, and sulfuryl fluoride (Singh and Sharma, 2015). Major stored product insects were found to be resistant to methyl bromide, including *Callosobruchus maculatus* (Fabricius), *C. chinensis* (Linnaeus), *C. analis* (Fabricius), and *C. phaseoli* (Gyllenhal) (Arora and Srivastava, 2021). Methyl bromide, which has negative impacts on both human health and the environment by significantly contributing to ozone depletion, has been used in a number of post-harvest control strategies for insects. Humans are susceptible to neurotoxic and cancerous consequences from methyl bromide (Bulathsinghala and Shaw, 2014) (Gautam et al., 2016). To control stored goods insect pests, efforts are being undertaken to create affordable, secure, and ecologically friendly alternatives (Navarro, 2006). Alternative approaches must be used, either as stand-alone applications or as "resistance breakers" in a rotation concept with methyl bromide. This is due to the rise in resistance populations around the world (Agrafioti et al., 2019).

Phosphine is an alternative approach for controlling *C. chinensis* in grains and other stored commodities (Collins et al., 2005). The grains are normally coated with the required amount of phosphine tablets or pellets and tightly sealed. These pills release deadly phosphine gas when in contact with moist air, which kills insects (Singh Gill and Singh Kolar, 1980). Traditionally, Botanicals are also used against pulse beetle to be quite safe and promising. However, the use of botanicals is one of the best options to overcome the problem of methyl bromide resistance (Singh. and Sharma, 2015). Botanicals and vegetable oils have insecticidal properties and growth inhibiting effects which can be used to keep the stored pulse free from pulse beetle attack (Kausarmalik and Rizwana, 2014). Neem and natural pyrethrum powder have the best control of pulse beetle (Paneru and Shivakoti, 2001). Boric acid has been used recently as a fertilizer, a non-selective biocide, and now used to control stored grain pests. It is also utilized as an antiseptic, for food-conservation and as a soil sterilent (Block, 2003).

The current study was designed to evaluate the efficacy of phosphine, boric acid and botanicals (Neem and Eucalyptus leaves) as an alternative, ecofriendly and economically feasible management of *C. chinensis*. This technique will help to define appropriate conditions of this physical pest control method for a better and safer management of *C. chinensis* and potentially other storage pests. This reduces the risk of contamination and ensures the quality of the stored grains.

MATERIALS AND METHODS

Area of Study

This experiment was conducted at the Insect Taxonomy Laboratory, Department of Entomology, Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan, Punjab, Pakistan.

Collection of Pulse Beetle

The specimens of the pulse beetle (*C. chinensis*) were collected from the damaged seeds of mung bean from local grain market located at Jahanian (30.0˚, 71.8˚), Khanewal Pakistan.

Rearing of Pulse Beetle

Collected insects were shifted to Taxonomy laboratory reared on fresh green grams (Gram dall) which contains 48% protein (24g) and 21% carbohydrate (63g) as a natural diet into plastic jars 1000-ml (Sarwar et al., 2020). These jars were covered with muslin cloth, tied with rubber bands under lab conditions 27 ±2°C and 65 ±5% R.H. It was done to provide proper aeration to the rearing insects but for maximum avoidance of insects to escape from the jars as well. The muslin cloths were fixed into the lids of the jars with rubber band. These jars were kept under the optimum conditions and the diet was added into jars after a two weeks interval. Female laid the eggs on grains. After 4-5 days eggs were hatched into newly larvae. These larvae separated with the help of aspirator and shifted into another jar which contains food material to get uniform generation. After 14-21 days larvae molts into dark brown pupae. Adults were emerged from pupae after 4 days.

Test Material

Phosphine tablets and boric acid were purchased from the local market (Kamal Pharmacy). Neem and Eucalyptus leaves were collected from the forest area of BZU Multan.

Experimental Setup and Design

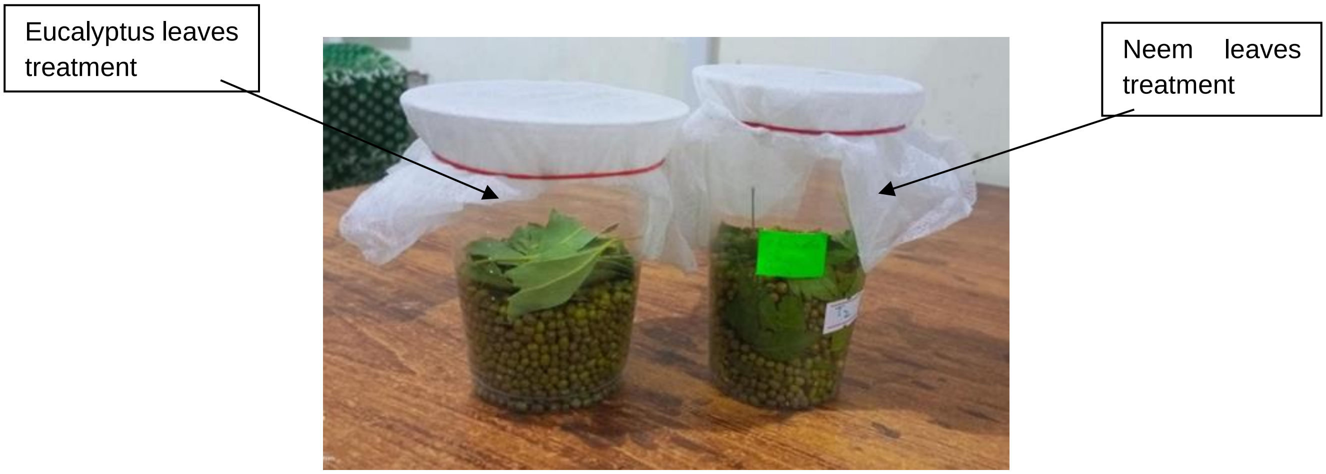
The study was conducted on food material under CRD with various concentrations of Phosphine and boric acid with five replications.

Bioassay of Phosphine and Boric Acid against Pulse Beetle

Plastic bottles (120 ml) were filled with 50g mung bean along with 20 adults of *C. chinensis*. Three concentrations (0.5, 1, & 2g) along with five replications of phosphine tablets and Boric Acid (0.06, 0.12 & 0.25g) were applied for 24, 48 and 72 hours respectively. Each bottle was sealed with rubber cork.

Toxicological Impact of Botanicals against Pulse Beetle

Plastic cups (250 ml) were filled with 100g mung bean along with 20 adults of C. chinensis. Neem and Eucalyptus leaves were collected and washed 2 to 3 times by fresh water. Leaves were dried in shaded places and applied in the cups with grains layer by layer. Each cup was covered with muslin cloth. Three concentrations 2,5 and 10g with five replications of botanicals were applied for 24, 48 and 72 hrs.



Mortality Percentage

Mortality percentage was calculated by using average and percentage formula (Sarwar et al., 2018).

$$\text{Average formula} = \frac{\text{Sum of all the Treatments}}{\text{No. of all the Treatments}}$$

$$\text{Percentage formula} = \frac{\text{Died Insects in treatment}}{\text{Total No of all insects in traetments}} \times 100$$

RESULTS

Bioassay of Phosphine on Pulse Beetle

The percentage mortality of pulse beetle was evaluated by using phosphine. The results indicated that the mortality percentage of pulse beetle varied at 24, 48 and 72h. The concentrations (0.5, 1 and 2g) of Phosphine showed maximum mortality 82, 87 and 96% after 24 hours, (Fig. 1) but the minimum mortality 82% was also occurred at 0.5g concentration of Phosphine So, concentrations were decreases as (0.06, 0.12 and 0.25g) but time duration was increasing as 48 to 72 h. After 48 h 27, 72 and 80% mortality were observed while after 72h with same concentration 45, 86 and 98% mortality were observed (Fig.2). The mortality was increasing as the time period was increased.

Bioassay of Boric Acid on Pulse Beetle

The percentage mortality of pulse beetle was evaluated by using boric acid. The results indicated that the mortality percentage of pulse beetle varied at 24, 48 and 72h. The concentrations (0.5, 1 and 2g) showed maximum mortality as 79, 82 and 91% after 24 hours, (Fig. 1) but the minimum mortality 79% was occurred at 0.5g concentration. So, concentrations were decreases as (0.06, 0.12 and 0.25g) but time duration was increasing from 48 to 72 h. After 48h, the mortality as 13, 44 and 72% was observed while on 72h at same concentration 18, 55 and 95% mortality were evaluated (Fig.2). The mortality was increasing as the time period was increased.

Bioassay of Neem Leaves on Pulse Beetle

The percentage mortality of pulse beetle was evaluated by using Neem leaves. The finding showed that the percentage mortality in pulse beetle increased with the increase in concentration of neem leaves and varied after 24, 48 and 72h (Fig3). The concentrations (2, 5 and 10g) showed different mortality at different time duration. After 24h the mortality was 18, 60 and 65%. As the duration was increased up to 48h the mortality was increased viz, 30, 77 and 90%. After 72hours maximum mortality 40, 80 and 96% at similar concentration was observed (Fig3).

Bioassay of Eucalyptus Leaves on Pulse Beetle

The percentage mortality of pulse beetle was evaluated by using Eucalyptus leaves. Results showed that the percentage mortality in pulse beetle increased with the increase in concentration of Eucalyptus leaves and varied after24, 48 and 72h (Fig3). The concentrations (2, 5 and 10g) showed different mortality at different time duration. After 24h the mortality was 17, 31 and 67%. As the duration was increased to 48h the mortality was also increased viz, 26, 42 and 86%. After 72hours maximum mortality 42, 60 and 95% at same concentration was observed (Fig3).

Table 1. Mortality percentage of phosphine and Boric acid at 24h, 48h and 72h.

Mortality %							
24 hours			48 hours			72 hours	
Dose	Phosphine	Boric Acid	Dose	Phosphine	Boric Acid	Phosphine	Boric Acid
2g	96	91	0.25g	80	72	98	95
1g	87	82	0.12g	72	44	86	55
0.5g	82	79	0.06g	27	13	45	18

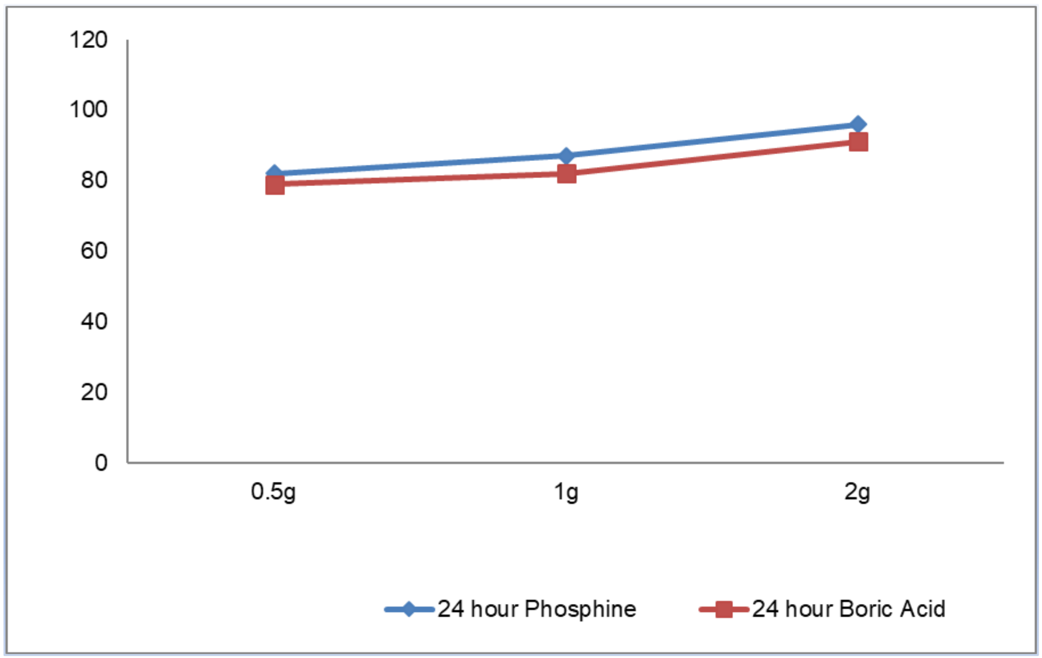


Figure 1. Mortality percentage of phosphine and boric acid at 24h.

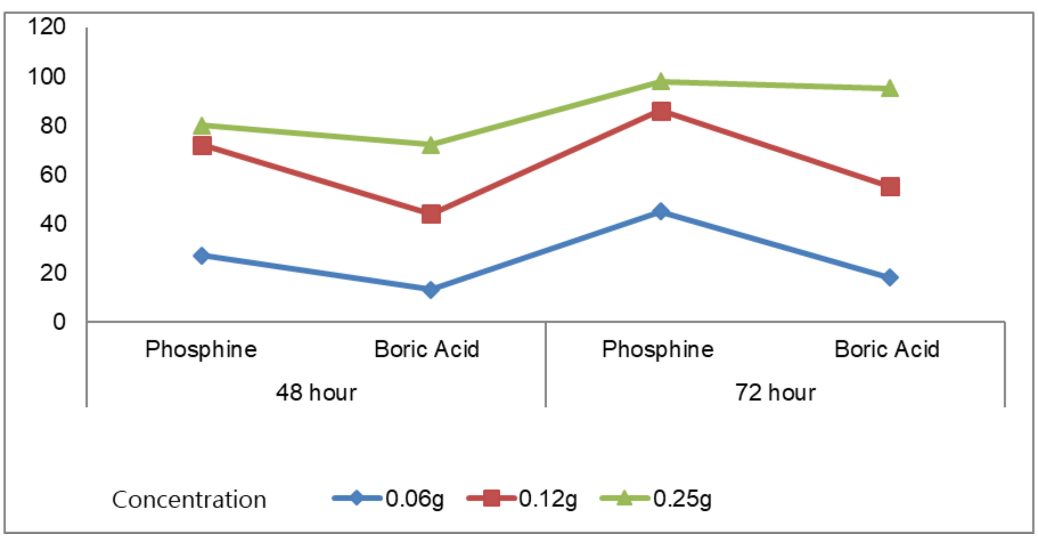


Figure 2. Mortality percentage of Phosphine and boric acid at 48h and 72h.

Table 2. Mortality percentage of Neem and Eucalyptus at 24h, 48h and 72h.

	Mortality %					
	24 hours		48 hours		72 hours	
Dose	Neem	Eucalyptus	Neem	Eucalyptus	Neem	Eucalyptus
10g	65	67	90	86	96	95
5g	60	31	77	42	80	60
2g	18	17	30	26	40	42

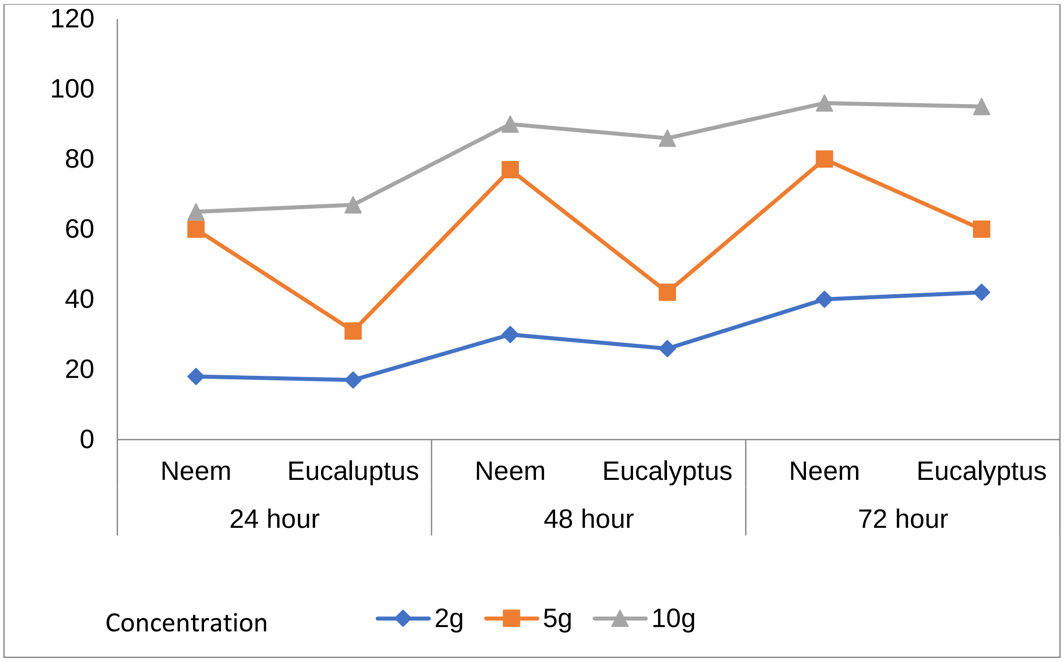


Figure 3. Mortality percentage of Neem and Eucalyptus Leaves at 24h, 48h and 72h.

DISCUSSION

Pulses are the most important sources of foods for human and may be stored for a long time. During this long storage period, many insect pests attack them, especially pulse beetle. This beetle is the most destructive pest of stored grains in adult and larval stages. Traditionally, many chemicals such as methyl bromide, methyl iodide were used for controlling the stored grain pest but most of the insect pests showed resistant against these pesticides and also have hazardous effect on consumer health (Wang et al., 2000). Use of phosphine, boric acid and botanicals are the best alternative technique to managing of pulse beetle.

In the present study the maximum mortality (98%) was observed due to phosphine at 0.25g after 72hrs. Already published study was reported by (Gandhi and Srivastava, 2019). *C. maculates* infested pulses were exposed to the phosphine. Maximum mortality (80%) was observed due to phosphine at 0.0125mg dosage in *Callosobruchus maculates* after 72hrs. The current study showed the maximum mortality (95%) at concentration of 0.25g of boric acid after 72hrs. Previous study was reported by (Malik et al., 2012). *T. castaneum* infested grains were exposed to boric acid and neem leaves. Maximum mortality (41and 25%) was evaluated for *T. castaneum* at 1g concentration after 72hrs.

In the present study the maximum mortality (96%) was observed due to neem leaves at 10g after 72hrs. Already published study was reported by (Ahmad et al., 2015) *C. chinensis* infested mung beans were exposed to the neem leaf powder. Maximum mortality 62% was observed of *C. chinensis* at 1.5mg after 72hrs. The current study showed the maximum mortality (95%) at concentration of 10g eucalyptus after 72hrs. Previous study was reported by (Kaur et al., 2019). *C. chinensis* infested pea seeds were exposed to the eucalyptus leaf powder. Maximum mortality 44% evaluated of *C. chinensis* at 5mg after 72 hrs. In the present study the minimum mortality (27%) was observed due to phosphine at 0.06g after 48hrs. Already published study was reported by (Arora and Srivastava, 2021). *Callosobruchus maculatus and C. analis* infested grains treated with phosphine. Minimum mortality 16% was observed at 0.0005 mg after 72hrs. In the current study the minimum mortality (27%) was observed due to boric acid at 0.06g after 48hrs. Previous study was reported by (Singh et al., 2022). *T. castaneum* infested grains treated with boric acid. Minimum mortality 33.3% was observed at 1.5g after 16 days. In the present study the minimum mortality (18%) was observed due to neem leaves at 2g after 24hrs. Already published study was reported by (Zafar et al., 2018) *C. chinensis* infested mung beans was exposed to the neem leaves. Minimum mortality 21% was observed at 0.5g after 24hrs.

In the current study the minimum mortality (17%) was observed due to eucalyptus at 2g after 24hrs. Previous study was reported by *C. chinensis* infested mung beans were exposed to the eucalyptus leaves. Minimum mortality 53% was observed at 2g after 15days. In the present study different concentrations of phosphine, boric acid and botanicals were evaluated against pulse beetle. The percentage mortality of insect pulse beetle increased with increase in time duration and concentrations of phosphine, boric acid and botanicals. Already published study was reported by (Hasan et al., 2006) who studied the response of *Trogoderma granarium* to different combinations of phosphine concentrations (0.1, 0.2 and 0.3g) and *Acorus calamus* oil doses (30, 50 and 70 μL). Percentage mortality was observed for different exposure periods of 3, 5 and 7 days. The results showed mortality of *T.granarium* increased that in combination with *Acorus calamus* oil and with increase in exposure period. In the present study results showed that the increase in the concentration of boric acid and the time duration the mortality also increases. Previous study was reported by(Kausarmalik and Rizwana, 2014). Mortality was increased in the increase in concentration of turmeric acid and time duration against *T. castaneum*. Another study reported by (Sarwar et al., 2020) Mortality effects of hypercapnia in modified atmospheres on larval and adult stages of red flour beetle that mortality increased with increase the concentration of CO2 level.

In the current study the increase in concentration and time duration of neemmortaality was also incerease. Already published study was reported by (Zafar et al., 2018). Mortality of *C. chinensis* increases with the increase the concentrations (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0%) of neem and different time duration (24, 48, 72hrs).

CONCLUSION

In the present study It is concluded that phosphine and boric acid are more effective against pulse beetle to reduce post-harvest losses in stored grains. The use of botanicals is also beneficial for the farmers and stockholders in both senses of availability and money. Use of these chemicals and botanicals are highly recommended as best pest managing techniques for stored grains.

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