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Effects of fermented Indigo-Leaf (*Indigofera tinctoria* L.) extracts on yield and pest control in Chinese mustard and sweet basil

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The purpose of this study was to investigate the impact on yields and pest control of extracts from fermented Indigo leaves (*Indigofera tinctoria* L.) on Chinese Mustard (*Brassica chinensis* Jusl var parachinensis (Bailey) Tsen & Lee) and Sweet Basil (*Ocimum basilicum* L.) For Chinese mustard, comparing crop yields revealed that fermented indigo-leaf extracts of mixed (tea-coloured and blue), tea-coloured and blue varieties gave yields of 24,444.44, 19,222.25 and 19,111.13 kg ha^{-1}, respectively. The fermented indigo leaf extracts of mixed (tea-coloured and blue) variety could be most appropriate for preventing and eradicating *Phyllotreta sinuata* Steph. For Sweet basil, fermented tea-coloured, blue and mixed (tea-coloured and blue) indigo leaf extract gave yields of 20,333.31, 17,833.31 and 17,555.56 kg ha^{-1}, respectively. The differences were statistically significant. The tea-coloured indigo leaf extracts caused decreased population of natural predator (*Menochillus sexmaculatus* (F.)) in basil plantation.

Key words: Indigo-leaf extracts, yield, pest control, Chinese mustard, sweet basil.

INTRODUCTION

In Thailand, insect pests such as striped flea beetles (*Phyllotreta sinuata* Steph.) infesting Chinese Mustard and maggots in Sweet Basil are important concerns for growing these vegetables. Insect pests have caused problems for large amounts of exported agricultural produce of many kinds. In expanding to grow large quantities of vegetables, a recurring problem in the growing plots has been that they are struck down by pests. Thus, the farmers need to use chemicals to prevent and eradicate pests, and produce that might be harvested before it is ready. Residues frequently accumulate in the vegetables in amounts over the standard values allowed. In addition, the use of such chemicals affects the environment and human wellbeing.

One possible way to improve this situation is by growing vegetables using extracts of certain herbs that possess more than one active insecticidal ingredient. The chance for insects to produce an anti-agent against such a mix of ingredients is likely to be slim (Wongthong and Pimsaman, 2005). The extracts experimented within this study were derived from *Indigofera tinctoria* L. There are rotenoids from *Isatis tinctoria* L. that have the quality of preventing and eradicating insect pests (Kamal and Mangla, 1993). Rotenoids can be found in *Derris elliptica* Benth (Sugsawat, 2005; Perry et al., 1998) which is highly active for killing insects by touch and sucking

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(Ahmed et al., 1983).

Derris elliptica Benth have low toxicity to humans, other vertebrate animals and the environment. It is highly toxic to fish and insect pests, and break down quickly when exposed to sunlight and high temperature (Tuncho, 2008). Rotenoids from *I. tinctoria* L. are mostly found in the leaves. There are six kinds of rotenoids, namely dehydrodeguelin, deguelin, rotenol, rotenone, tephrosin and sumatrol (Royal Project, 2008).

A study of *I. tinctoria* L. should be conducted, especially with respect to controlling insects that are pests of vegetables grown for domestic consumption and export. Success in such studies could lead to a new way to bring domestic plant resources to their full potential and sustainability.

MATERIALS AND METHODS

Preparation of fermented extract from indigo leaves

Four kilograms of fresh leaves were put in a 50 L plastic container. The tank was then filled with 10 L of freshwater. The leaves were pressed to submerge them and soaked for 24 h. Then the dregs were separated out of the tank and the extract was filtered. There were microorganisms especially *Bacillus alkaliphylus* in aeration and indigo leaves (Chanayati, 2001). The filtered extract was divided into 2 parts. The first 5 L of the indigo-blue extract (indoxyl and glucose with acidity pH 4.8) (Chanayati, 2001) was employed for experiments in vegetable plots. The remaining 5 L of the fermented indigo extract was mixed with 100 g of lime in tap water. The fermented indigo extract was stirred until it became dark blue, then it was left for 24 h until the residue had sunk down to the bottom of the tank. The tea-coloured indigo extract was then poured out of the upper tank for experiments in vegetable plots. The tea-coloured indigo extract had substantial amounts of nutrient, lime and active rotenone and others (Preparation of fermented extract from indigo leaves by Local wisdom methods in Sakon Nakhon province)

Experiments to examine the effects of fermented indigo leaf extracts on yields and pest control in Chinese mustard and Sweet basil

Examination of the effects of the fermented indigo-leaf extracts on Chinese mustard and Sweet basil using a randomized complete block design (RCBD) comprised 4 treatments and 3 blocks: Treatment 1 (tea-coloured fermented extract), Treatment 2 (indigo-blue fermented extract), Treatment 3 (tea-coloured and indigo-blue mixed together), and Treatment 4 control (tap water) were paired with vegetables. Seedlings were raised in seed pans containing soil: compost mix (1:1 v/v). After germination the seedlings were maintained for two weeks. When the seedlings aged 15 days, they were transplanted into the seedling bag. After seedlings were maintained for 15 days, they were brought to grow in the growing plot. The growing plot size was 1.5×2 m^2 and elevated as high as 15 cm. Bogashi compost (hull and manure mixed together for 30 days), 5 kg/plot, was added after periods of 15, 30 and 45 days.

The tea-coloured fermented indigo extract was used to water the vegetables for prevention and eradication of insect pests in a ratio of 25 ml/10 L of water. The indigo-blue fermented extract was also used in a ratio of 25 ml/10 L of water. The tea-coloured and indigo-blue extracts were also mixed in a ratio of 25: 25 ml/ 10 L of water, and tap water was used as a control. All extracts were prepared for watering every one week. Absolute method was used. A yellow sticky trap was used and the insects were counted on the trap. Notes were taken by observation of the kinds of insect found; the numbers of insects found on vegetables before and after one week of watering with each extract were also counted. Chinese mustard was harvested 50 days and Sweet basil 55 days after planting in the plots. The quantity of each kind of vegetable produced per plot was weighed. The crop yields with each treatment were weighed, recorded and analysed and compared using analysis of variance; a pair wise comparison for a difference of means was done using Duncan's New Multiple Range Test (DMRT).

RESULTS AND DISCUTION

Investigating the effects of fermented indigo leaf extracts on yields of Chinese mustard

In comparing which fermented indigo leaf extract had more effects on yields of Chinese mustard with the three experiment which used the tea- coloured fermented extract, the indigo blue fermented extract and mixture of both, it was found that treatment using the mixture resulted in the highest yield, an average of 24,444.44 kg ha^{-1}. This average weight was significantly different from the yields obtained from using either the indigo tea-coloured fermented extract or the indigo blue-coloured fermented extract alone, which produced 19,222.25 and 19,111.13 kg ha^{-1}, respectively, ($p<0.05$); whereas tap water (control) yielded only an average of 12,777.75 kg ha^{-1}, (Figure 1). The indigo tea- coloured fermented extract was appropriate for Chinese mustard, yielding 24,444.44 kg ha^{-1}

It was evident that the yield of Chinese mustard using the mixture of both extracts was the highest, while using the tea-coloured extract and indigo-blue extract individually, produced successively lower yields, although still higher than the control plots. This is because as a plant of Papilionaceae, Indigo's root nodules carry *Rhizobium indigoferae* which have nitrogen-fixing capacity (Garrity et al., 1994). The plants, then, include food minerals that are essential for their growth. Their leaves had a high amount of nitrogen. Therefore the fermented indigo-leaf extracts had quantities of highly concentrated organic minerals. In a particular indigo leaf, the level of nitrogen was as high as 5.11% as well as diphosphorus penta-oxide 0.78% and potassium oxide 1.68% and calcium from lime (Royal Project, 2009; Tuncho, 2008; Reangrug and Tuntiwat, 1991). The Chinese mustard, then, could grow better.

The effects of fermented indigo-leaf extracts on pest control in Chinese mustard

During investigation of widespread insect pests in the Chinese mustard plots before use of the fermented indigo extract, striped flea beetles were found in the plots before the treatments on average 15.33, 15.67, 15.67 and 16.67

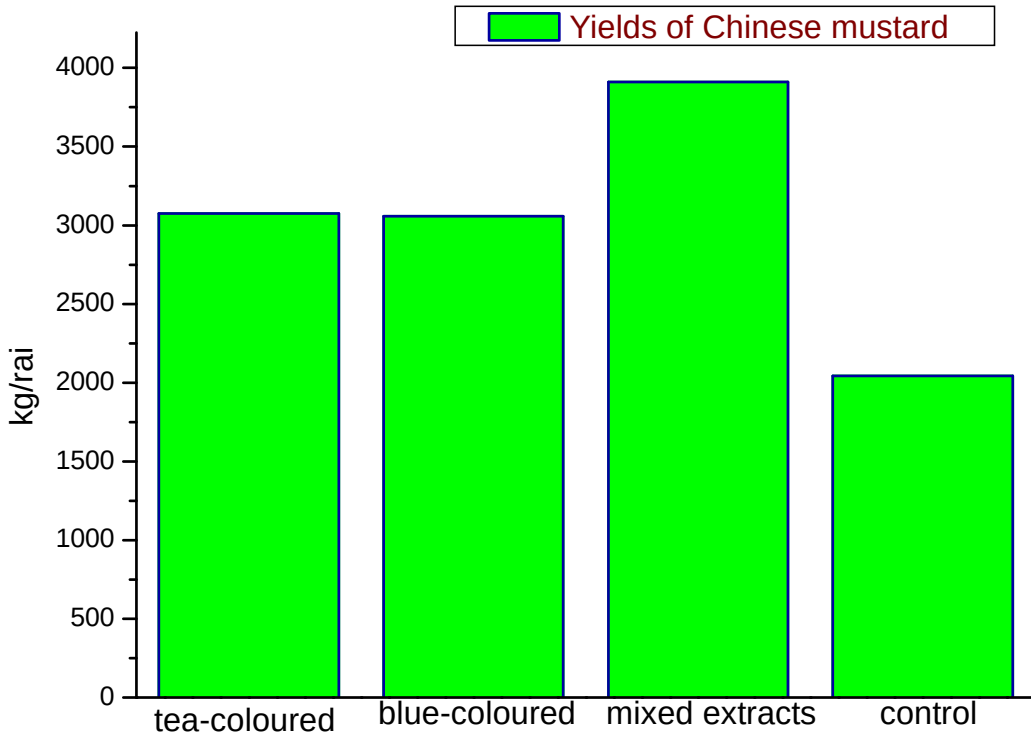

Figure 1. Comparing the yields of Chinese mustard with the tea-coloured, blue and mixed (tea-coloured and blue) fermented indigo-leaf extracts, and control.

Table 1. Average number of *P. sinuate* Steph. in Chinese mustard

Treatment	Average number of *P. sinuate* Steph.					
	Before treatment	1 week	2 weeks	3 weeks	4 weeks	5 weeks
Tea-coloured indigo extract	15.33	3.33^{a}	1.00^{a}	2.33^{a}	1.67^{a}	2.00^{a}
Blue-coloured indigo extract	15.67	9.67^{b}	8.00^{b}	3.67^{a}	2.33^{a}	3.33^{a}
Mixed of both	15.67	0.33^{a}	0.00^{a}	1.33^{a}	0.67^{a}	0.67^{a}
Control	16.67	19.33^{b}	12.67^{b}	10.67^{b}	8.33^{b}	8.00^{b}
F-test	ns	*	**	*	*	*

Note: Means in each column followed by the same letter are not significantly different . ($P < 0.05$) from each other according to DMR test.

fleas/plot were noted. There were no significant differences between the average numbers of striped flea beetles. By analysis of variance after the first watering after 1 week with the three treatments, it was found that the average number of striped flea beetles on the Chinese mustard growing plots showed significant differences ($p<0.05$) among all the treatments. After watering the Chinese mustard using the tea-coloured and indigo-blue mixed together, striped flea beetles were minimal, occurring on average only 0.33 flea/plot. After 2 weeks of watering the Chinese mustard with, the tea-coloured and indigo-blue mixed together and the indigo tea-coloured fermented extract, the average number of striped flea beetles for each treatment showed highly significant differences ($p<0.01$).

The striped flea beetles were not found in the growing plots that used the tea-coloured and indigo-blue mixed together, whereas in the plots with the indigo tea-coloured fermented extract, an average of only 1.00 flea/plot was found. After 3 to 5 weeks of watering with the indigo tea-coloured fermented extract, the indigo blue-coloured fermented extract, the tea-coloured and indigo-blue mixed together and control, the average numbers of striped flea beetles for each treatment were significantly different ($p<0.05$) shown in Table 1. In a nutshell, watering the fermented indigo extract 1 to 2

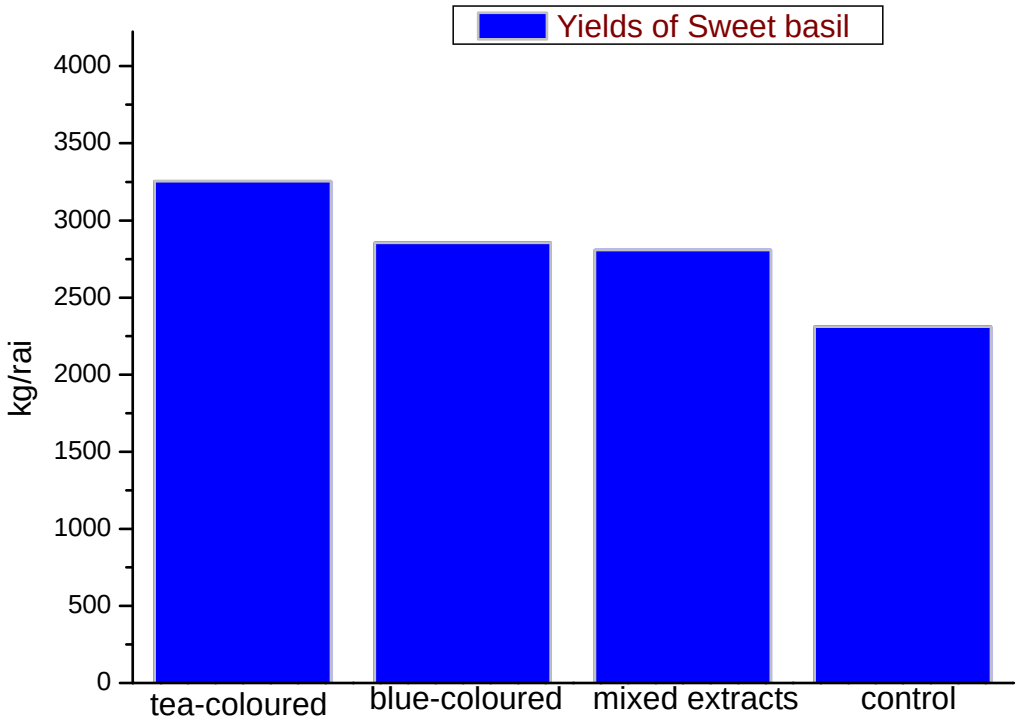

Figure 2. Comparing the yields of Sweet basil with the tea-coloured, blue, and mixed (tea-coloured and blue) fermented indigo-leaf extracts and control.

weeks could control number of striped flea beetles in Chinese mustard plots.

It was apparent that watering the Chinese mustard with indigo-blue and tea-coloured fermented extracts could be beneficial for prevention and eradication of insects. Since each kind of extract from the fermented indigo-leaves contained rotenoids, which have several active ingredients and an acidic quality, watering the Chinese mustard growing plots on which the striped flea beetles larvae and their matured maggots live by day with the fermented indigo-leaf extracts resulted in visible decreases in striped flea beetles after just one or two times.

The fermented indigo-leaf extracts could be used to prevent and eradicate insects that are pests of these vegetables (Wongthong and Pimsaman, 2005; Kamal and Mangla, 1993; Sugsawat, 2005; Royal Project, 2009; Reangrug and Tuntiwat, 1991). Using the fermented indigo-leaf extracts to water or spray the Chinese mustard 2 or 3 times with an interval of 5 to 7 days between each treatment reduced the numbers of striped flea beetles. Thus, fermented indigo-leaf extracts could be useful for controlling pest insects of these vegetables. The use of a mixture of tea-coloured and blue-coloured fermented indigo-leaf extract could produce the highest yield and most effective control of striped flea beetles in Chinese mustard followed by the tea-coloured and the blue-coloured fermented indigo-leaf extract, respectively.

The effects of the fermented indigo-leaf extracts on Sweet basil yields

When the effects of using the three kinds of fermented indigo-leaf extract were compared, it was found that the yields of Sweet basil with and without receiving treatment by the fermented indigo-leaf extracts showed a significant difference ($p<0.05$). Sweet basil treated with the tea-coloured indigo fermented extract produced the highest yield of 20,333.31 kg ha^{-1}on average; below this was the blue indigo fermented extract, the tea-coloured and indigo-blue mixed together and the control, with yields of 17,833.31 17,555.56 and 14,444.44 kg ha^{-1}, respectively, Figure 2. The tea-coloured indigo fermented extract was appropriate for Sweet basil, yielding 20,333.31 kg ha^{-1}

It was evident from the treatments that the Sweet basil watered with the tea-coloured fermented indigo-leaf extract produced the highest yield, and below this ranked the treatment with the indigo-blue extract and the treatment with a mixture of both extracts. This was because the fermented indigo-leaf extracts had high concentrations of organic elements and minerals, especially nitrogen, as high as 5.11% and high calcium from lime (Royal Project, 2009; Reangrug and Tuntiwat, 1991). That resulted in more growth of Sweet basil, the tea-coloured fermented indigo-leaf extract was suitable for watering garden vegetables for increasing their growth and keeping them free of disturbing insects.

Table 2. Average number of *M. sexmaculatus* Fabr. in Sweet basil.

Treatment	Average number of *M. sexmaculatus* Fabr.					
	Before treatment	1 week	2 weeks	3 weeks	4 weeks	5 weeks
Tea-coloured indigo extract	10.67	2.67[a]	1.67[a]	1.33[a]	2.00[a]	1.33[a]
Blue-coloured indigo extract	15.33	5.00[b]	3.67[a]	2.33[a]	1.67[a]	2.00[a]
Mixed of both	14.67	2.00[a]	2.00[a]	1.00[a]	0.67[a]	1.33[a]
Control	16.33	5.67[b]	6.33[b]	4.67[b]	5.33[b]	4.67[b]
F-test	ns	*	*	*	*	*

Note: Means in each column followed by the same letter are not significantly different ($P < 0.05$) from each other according to DMR test.

The effects of the fermented indigo-leaf extracts on insect control in sweet basil

From the results of counted insects found on the growing plots of Sweet basil, *M. sexmaculatus* Fabr. (Ladybird beetles) were found with a serrated pattern on their wings. Before watering the plants with the treatments, the counts of ladybird beetles on the plots of all the treatments intended to receive the tea-coloured indigo fermented extract, the blue indigo fermented extract, the tea-coloured and indigo-blue mixed together and the control plots showed the average numbers of 10.67, 15.33, 14.67 and 16.33 counts/plot, respectively. There were no significant differences between the average numbers of ladybird beetles.

By analysis of variance after first watering after 1 week with the three treatments, it was found that the average number of ladybird beetles on the Sweet basil growing plots showed significant differences ($p<0.05$) among all the treatments. After 2 to 5 weeks of watering with the tea-coloured fermented extract, the blue-coloured fermented extract, the tea-coloured and indigo-blue mixed together and the control, the average number of ladybird beetles showed significant differences ($p<0.05$), Table 2. In a nutshell, watering the fermented indigo leaf extract for 2 weeks had significant effects on the population of natural predator (*M. sexmaculatus* F.).

It was evident that with treatments of the tea-coloured extract itself, or a mixture of it with the indigo-blue extract just one or two times, the number of ladybird beetles decreased. It showed that the fermented indigo affected a number of nodes because in indigo leaves there are rotenoids which are active ingredients in preventing and eradicating insect pests of plants (Wongthong and Pimsaman, 2005; Kamal and Mangla, 1993; Sugsawat, 2005; Royal Project, 2009; Reangrug and Tuntiwat, 1991). This was in congruence with Perry et al. (1998); Ahmed et al. (1984) that in Indigo leaves, there are 6 rotenones which affects the number of nodes by contact. Thus, control over ladybird beetles on the Sweet basil plots using the mixture of tea-coloured and indigo-blue fermented indigo-leaf extracts or using the tea-coloured extract alone to spray two or three times on the plants with an interval of between five and seven days before the next spray resulted in a decreased number of ladybird beetles. This was in congruence with the Department of Agriculture which noted that substance rotenoid affects ladybird beetles. The fermented indigo leaf extract of tea-coloured and blue causes decrease in the population of natural enemies (*M. sexmaculatus* Fabr.) in Sweet basil plot. This was in congruence with Teanglum (2013) who noted that the fermented indigo leaf solutions of tea-colored and blue-colored extracts decreases the population of natural enemies (*Menochilus sexmaculatus* (F.)).

Conclusion

Application of the tea-coloured and indigo-blue extracts mixed together for prevention and eradication of *Phyllotreta sinuata* Steph on the Chinese mustard plots resulted in the highest yield, 24444.44 kg ha^{1}. Below this, were yields of 19222.25 and 19111.13 kg ha^{-1} obtained by using only the tea-coloured or the indigo-blue extracts alone, respectively. The tea-coloured fermented indigo extract gave the highest yield on Sweet basil, equalling 20333.31 kg ha^{-1}. Below this were Sweet basil yields of 17833.31 and 17555.56 kg ha^{-1} obtained by using the indigo-blue extract and the mix of both extracts, respectively. The tea-coloured and blue fermented indigo-leaf extracts caused a decrease in population of natural enemies (*Menochillus. sexmaculatus* Fabr.). In a nutshell, the application of extracts from indigo leaves for control of insect pests on vegetable plots is helpful for decreasing chemical residues in vegetables.

Conflict of Interests

The authors have not declared any conflict of interests.

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